ANNUAL REPORT
OF
PROGRAM ACTIVITIES

NATIONAL INSTITUTES OF HEALTH

1962

NATIONAL INSTITUTE OF DENTAL RESEARCH

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NATIONAL INSTITUTE OF DENTAL RESEARCH
Annual Narrative and Project Reports
Calendar Year 1962

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THE ANNUAL REPORT OF THE DIRECTOR

The National Institute of Dental Research

Calendar Year 1962

by

Dr. F. A. Arnold, Jr.

As the National Institute of Dental Research completes its 15th year, current research activities in both the intramural and extramural programs are being critically reviewed to determine their productivity.

In carrying out the research mission of the Institute, close working associations with the faculties and administration of dental schools, research institutions and the leaders of the dental profession are essential.

With the continual growth of the Institute, there is a constant need to strengthen staff resources in the areas of program planning and general administration. In order to develop information required for an effective evaluation of activities and provide sound program development, an office of Program Planning was established within the Office of the Director. This staff component has devoted most of its time to the extramural program area where the need for a complete program evaluation is most urgent.

As the number of personnel engaged in dental research continued to expand, closer control over the general administration of the Institute became necessary. This resulted in the adoption of numerous procedures in order to gain a more accurate and closer understanding of the following problem areas: (1) Appointments to Advisory Groups and Committees; this is used as a central reference source for procedures and instructions to follow for such appointments; (2) A general implementation of established procedures was also prepared to assist personnel in the objective and purpose of official travel in connection with the Institute's projects; (3) A general informative document covering training of employees is now in preparation. This will enable each employee to familiarize himself with the types of training available, restrictions, and guidance in the proper preparation of applications; (4) A systematic follow-up program was developed to insure timely and accurate preparation and submission of all types of administrative reports. For all practical purposes, this system has virtually eliminated the waste and inaccuracies usually accompanied by hasty preparation of recurring reports.

Space continues to be a problem of major significance and in certain areas has become quite critical. Acquisition of additional space has been requested.

During the year, the following changes were made in the organizational structure of the Institute: (1) A section on Clinical Dental Services was established in the Clinical Investigations Branch. This assures the close coordination of the many overlapping activities in handling the patient care services of the Clinical Center for effective study of patients in connection with research projects; (2) A Section on Crystal Chemistry was established in the Laboratory of Histology and Pathology. The staff assigned to this activity will be engaged in the study of the structure and properties of mineralized tissues.

Intramural Research

In the direct research program of the Institute, the major studies now being emphasized include: (a) Development of newer, recently advanced, concepts of dental caries etiology using the germfree animal technique; (b) Studies of periodontal disease including the relationship of general systemic factors to disease and the causal significance of various microbic and enzymatic factors; (c) The epidemiological patterns of dental disease, especially in underdeveloped countries; (d) The mechanisms of calculus formation and the relationship of calculus to oral disease processes; (e) Research on cleft palate and other congenital anomalies; and (f) Research on the growth and development of the oral and facial regions.

The importance of basic research in dentistry and the exploration of disease states in which the oral component is a significant factor have underlined the contribution of NIDR scientists to a wide range of studies from genetics to malignant tissues. A recent study of our direct operations revealed that approximately 60% of our principal investigators are engaged in collaborative research with other Institutes or with other research institutions, both governmental and non-governmental. This high percentage of collaboration is greater than in other Institutes and is particularly significant in that it portrays the broad approach taken in achieving our research goals.

Worthy of note was the Scientific Award of the Albert Joachim International Prize to Drs. Paul H. Keyes and Robert J. Fitzgerald on July 7th at the Annual Meeting of the Federation Dentaire Internationale in Cologne, Germany. The prize is awarded every five years for the best original work on scientific research in the field of odonto-stomatology. Their research findings were related to the infectious nature of experimental caries and the cariogenic potential of streptococci. The techniques developed in these studies are being adopted to research on the role of bacteria in periodontal disease.

Also during the year 54 NIDR manuscripts appeared in the journals and an additional 84 were accepted for later publication.

Extramural Research

Within the extramural program, there is an increasing emphasis on the categorical missions of the Institute. There are now 455 research grants in operation in 138 research institutions situated in 36 states and 13 foreign countries.

The programs relate to a broad spectrum of research in the causes, treatment, and prevention of oral disease.

Emphasis this year has been on expansion of research activity in cleft palate, congenital anomalies, periodontal disease, and studies in the sciences basic to dentistry and medicine.

During the past year, the staff, with the assistance of advisory groups, thoroughly reviewed the Training and Fellowship programs. The studies revealed deficiencies in the concept and operation of existing Training Programs and shortage of training opportunities. The following recommendations were made: (1) Emphasis will be placed in the development of Ph.D programs; (2) Lengthening the period of research training in the clinical disciplines; and (3) Providing more time for research training and experience in addition to the time required for the development of clinical skills. Guidelines for improving these programs were recommended and steps are currently being taken to implement these recommendations.

Meetings have been held with the dental school deans to carry out these recommendations. Future plans call for expansion in the basic sciences as they relate to dentistry at the predoctoral level in graduate schools.

There has been a substantial increase in the authorization of Research Career Development Awards. While the National Advisory Dental Research Council has not yet approved any Career Awards, it is anticipated that a small number of such awards will be made during the coming year.

This year marks the first time that funds have been appropriated for Clinical Research Centers. Preliminary guidelines for the establishment of dental clinical research centers were developed during the year and preliminary plans were formulated for initiating programs at several institutions. It is our thinking at this time that the majority of the funds allocated this fiscal year for this purpose will be utilized to establish pilot-type grants in this area. Also, emphasis has been placed on establishing a few broader research programs which may later require clinical facilities.

Currently under study are plans to develop Dental Research Institutes or Centers in a few large university environments. Four dental research sites believed to possess the highest potential for the establishment of such institutes have been selected. During the coming year, it is assumed that some of these programs will become operational. It is anticipated that the major problems encountered will be: (1) securing institutional matching money for facilities construction; and (2) the recruitment qualified personnel at salary levels which prevail at dental centers.

THE ANNUAL REPORT OF THE EXTRAMURAL PROGRAMS BRANCH

The National Institute of Dental Research

Calendar Year 1962

by

Dr. F. Earle Lyman

Research Grants Program

During the year research sponsored by the grants program has been devoted to most of the major areas of interest to dentistry. Among these are: adhesive restorative materials, cleft palate and other facial congenital anomalies, periodontal disorders, calcification, caries, growth and development, and epidemiological studies of oral diseases. In addition to clinical investigations, efforts have continued in those other aspects of dental research such as basic science studies related to oral health, dental public health, and studies to improve dental materials.

All 49 dental schools in the United States have now participated in the research grant program. Approximately 75 percent of the funds allocated for research projects are devoted to research support at these institutions and closely related dental clinics. During 1962, the number of active research grants totalled 450 at 138 institutions in the United States and 13 foreign countries. The amount of dollar support has increased $1,073,000 due to the establishment of new broad interdisciplinary program projects.

Indicative of the increased breadth of dental research today are plans for developing three or four dental research institutes in centers of higher education such institutes to be designed to carry on broad-based clinical and basic research. Adequate facilities to house personnel and equipment are projected, and graduate training and career development programs are included in the planning for these institutes. To implement this program as rapidly as possible, dental research sites believed to possess the highest available potential for the establishment of such institutes have been selected. In addition, it is felt that the long-range goals of the program should include the establishment of dental research institutes in non-dental environments, and preliminary discussions have been held toward this end.

Continuing interest in the cleft palate field is shown in the approval of three applications for cleft palate centers ranging in size up to several hundred thousands of dollars at the University of Pittsburgh, Eastman Dental Dispensary, and the Variety Children's Hospital in Miami. Support is increasing for basic research utilizing a team approach in the medical, dental, and social disciplines to augment our understanding of this anomaly and to provide a better basis for optimal rehabilitation.

Prominent this year in the field of congenital and speech anomalies was the award of a grant, to the Eastman Dental Dispensary, in support of a research program to measure simultaneous significant parameters of normal speech sound productions after pharyngeal flap operations have been performed. In addition, comparative analyses of operative and pre- and post-operative data will differentiate relative success or failure and will identify factors respectively associated.

An interdisciplinary program-project on periodontal disease was awarded to scientists at the Eastman Dental Dispensary. Abnormalities of tooth contact have long been recognized as contributing to the breakdown of the periodontal tissue, but up to this time no comprehensive study of the subject has been undertaken to include not only the contact between individual teeth but the relationship of the whole complex of jaw movement in relation to the forces and pressures bearing on individual teeth. These investigators have been able to build devices into the crown of teeth which, upon direct contact, transmit an impulse by radio which can be picked up by a receiver at quite some distance. This technique allows the recording of the number of contacts on a 24-hour basis and the length of the contact. It is proposed to relate these findings to the state of health of the periodontium. Additionally, tissue response to injurious influence will be studied in this project.

During the year a $1,000,000 program project was approved which will support a seven year study at the University of Minnesota on a multifaceted investigation in the field of the biochemistry and physiology of the fluoride ion. More knowledge of the routes and factors affecting the transports of fluoride into and from calcified tissues, and the effects of the ion on these tissues, might point out the mechanism of fluoride inhibition of caries. Such information might suggest agents superior to fluoride for use in caries control.

A significant study providing additional evidence that low dental caries experience of children who consume fluoridated water from birth persists during adult life has been conducted by NIDR grantee investigators at the College of Dentistry, University of Illinois. These investigators have reported on the first study conducted in the United States in which the dental caries experience of adult natives of a city with an optimum concentration of naturally fluoridated water (Aurora, Illinois, 1.2 ppm) was compared with that of adult native residents of a nearby fluoride-deficient city (Rockford, Illinois, 0.1 ppm). Other U. S. studies have reported on persons using either heterogeneous or higher levels of fluoride in communal water supplies.

During the year a new grant was approved which will support an epidemiological study on fluorine: a salt fluoridation study in four Columbian communities. This study seeks to determine the effectiveness of common salt as a vehicle in fluoridation programs for caries prevention. The effectiveness of sodium and calcium fluoride as salt additives in caries prevention will be compared. This study has special significance because of the fact that 100 million people of the Americas have access to water supplies unsuitable for fluoridation.

New findings this year at the Forsyth Dental Infirmary have shown the extreme importance of the surface enamel in regard to protection against caries. Two minor enamel constituents, fluoride and carbonate, play major roles in the surface portions of enamel, an increase in fluoride concentration increases caries resistance, and an increase of carbonate decreases caries resistance. The most promising approach now available in controlling caries consists of modifying the chemistry of the enamel surface, as is accomplished in water fluoridation and topical fluoride treatments.

The surface of enamel differs in composition from underlying enamel in many respects. It is slightly more calcified than inner enamel and has higher concentrations of fluoride, zinc, lead and certain other trace elements, and lower concentrations of carbonate, sodium and magnesium. Some trace elements, including strontium are evenly distributed in surface and subsurface enamel. The accumulation of fluoride and other "skeletal seekers" in the surface enamel begins as soon as the enamel has calcified, and continues posteruptively throughout life. The concentrations of fluoride in surface and subsurface enamel are closely related to the level of fluoride in the drinking water. Contrary to fluoride, the carbonate in surface enamel decreases in concentration with age.

In a collaborative epidemiological study, the oral health of Icelanders has been surveyed by the University of Alabama - University of Iceland team of grantees. A pilot study involving more than 600 children ages 2 to 7 has been completed. Evidence indicates this group has a higher prevalence rate of tooth decay than observed in comparable nations. Gingivitis was encountered more commonly in Icelandic children than in their American, English, and Danish counterparts. The prevalence of malocclusion was similar to that in Danish children, but lower than in American and English children. Dental anomalies including supernumerary teeth and congenitally missing teeth were a frequent occurrence.

Also in Iceland, a carefully designed random study of persons 6 years and older is nearing completion. The examination standards are comparable to those established for the United States National Health Survey. Four thousand persons, 2½ percent of the general population, are included in the sample. It is the impression of the investigators that the prevalence of dental caries in Iceland exceeds that of any other country. Factors responsible for this situation are being evaluated. A dental examination of Icelandic skulls has been undertaken by collaborators, who report that dental decay was almost absent in Iceland one hundred years ago.

A genetic study on the etiology of cariogenesis has been pursued by investigators at Bowman Gray School of Medicine. Major genes have been identified which affect the formation of dentin and enamel but these uncommon defects account for only a minority of patients with caries. The susceptibility or resistance of the majority of patients is also genetically regulated, probably by a polygenic system. This inference is supported by both twin studies and by pedigree analysis and it may be expected that the frequency of caries in children will partially reflect the caries frequencies of their parents. Further research is required in this area to identify factors having possible clinical application.

A grant resulting from the concerted efforts of the Dental Study Section and the Council to stimulate research in a basic area essential to a better understanding of oral health will support a conference on trigeminal anatomy and physiology, to be held at the Mayo Clinic, Rochester, Minnesota. The proceedings of this conference will be published, including a comprehensive review of the current knowledge in the field, to be used as a point of departure to stimulate research in this area and to broaden understanding of the fifth cranial nerve.

The University of Arizona has developed a technique for determining chemical elements in dental tissues by the use of monochromatic x-rays for microradiology. By using the technique of x-ray adsorption chemical elements of dentine and other tissues can be identified and the morphology of a substance outlined as indicated by the differences in density of the substance. It is expected that this type of examination can contribute much to the understanding of the processes of calcification and decalcification.

The Institute this year has continued to support a study on the development of dental adhesives at the Eastman Dental Dispensary, of Rochester, New York. A plan of cooperation was drawn up with the Monomer-Polymer Division of the Borden Company. A number of promising compositions are reported which show potential as dental materials. This potential involves their use as temporary and permanent restoratives, for cementing and for pulp capping agents. During the course of the present grant, two separate studies were also completed and published. One study was directed at obtaining information on both the ability of available commercial and experimental filling forming materials to adhere to tooth structure and on their permeability. The second study involved an evaluation of the effect of various bases and cavity liners on marginal leakage of dental restorations.

Investigators at the Polytechnic Institute of Brooklyn, New York, have been awarded a grant in support of a program of study leading to the development of superior polymeric dental adhesives. An attempt will be made to prepare a relatively soft, elastic liner with adhesive properties toward tooth and filling, thus acting as a cement which would absorb the thermal stresses. Tracer techniques would be employed in studies of organic synthesis, absorption, and adhesion. A special instrument will be built for measuring wetting angles. If this project is successful a vastly superior filling material would be available to clinicians.

The National Advisory Dental Research Council, recognizing the contributions to be made, recommended the awarding of grants to scientists in foreign countries in support of unique and outstanding research projects. Twenty-three such grants amounting to approximately $232,000 were activated during the calendar year.

In summary, during the calendar year 1962 the Extramural Programs Branch, National Institute of Dental Research, received 339 research grant applications requesting $9,400,000 for the first year of support. Of these, 180 applications were approved for a sum of $4,100,000. Council also approved the payment of 268 previously committed grants in the amount of $4,100,000.

Training Grant Program

The NIDR Graduate Training Program is concerned with assisting qualified public and other non-profit institutions to establish, expand, and improve training opportunities in order to develop a larger reservoir of competent research investigators and teachers in the clinical and basic science disciplines of importance to dentistry. The program is designed to meet the critical shortage of research and academic personnel presently available to dental schools and other research institutions. The need for additional research personnel in the dental areas is constantly increasing, thus the training program must constantly grow to meet this demand.

The sum of $3,527,000 has been appropriated for training grants from fiscal year 1963 funds. It is estimated that these funds will support approximately 55 training centers and 350 trainees. Currently there are 55 active training grants supporting approximately 198 trainees at 44 training institutions. These centers are located in 22 States and the District of Columbia. During the calendar year 1962, 13 new grants were activated. In addition, 8 training centers received supplemental grants.

In order to strengthen training in the basic sciences and place more emphasis on training toward the Ph.D. degree, applications from basic science departments not affiliated with dental schools have been stimulated and received. Prior to and during 1962 only a very few such programs were supported by NIDR. During 1962 approximately twelve such programs were reviewed and approved for activation in 1963. These include programs in metallurgy, colloid chemistry, crystallography, and genetics, all of which are areas where serious manpower shortages exist in dental research.

Also in 1962, NIDR has formulated plans to initiate special clinical research training programs in a few schools in the near future. Under such a program a small number of dental students would be carefully selected at the end of their sophmore year for specific clinical research training which would include both a Ph.D. degree in a basic science and board qualifications in a dental specialty. The period of training would cover a minimum of six years including summers beyond the two years of preclinical training and would allow basic science, research methodology, and clinical training to be taken concurrently.

Fellowships Program

A. Regular Research Fellowships

The primary purpose of the research fellowship program is directed toward training support for qualified scholars for research and academic careers in those health related basic and clinical sciences of interest to dentistry. Support is provided to the individual with special emphasis being placed on training in those basic science areas such as biochemistry, physiology, histology, and genetics which are related to the study of periodontal disease, cleft palate and oral disorders.

During 1962, a total of $636,952 was awarded for 87 regular fellowships at 41 institutions located in 24 states, the District of Columbia, and 1 foreign country. In comparison, last year 71 regular fellowships were awarded for a total of $521,299.

Also during 1962, a move was begun to place greater emphasis on the predoctoral fellowships supported by NIDR. It is hoped to recruit more scholars under this type of award, who are not holders of the D.D.S. degree, for training toward the Ph.D. in basic science departments not generally associated with the dental schools. These persons' training might, nevertheless, involve research projects related to dentistry, thereby creating an interest in pursuing dental research.

B. Research Career Awards

Research Career Awards are available to institutions in the United States on behalf of qualified candidates to provide increased numbers of stable career opportunities for scientists of superior potential and capability in the sciences related to health. Awards are intended to provide individuals with some measure of assured support reasonably early in their careers. Two groups of awards are available as follows:

1. Career Awards are made to enable institutions to finance stable positions favorable to the intellectual growth and research productivity of established investigators of high competence for the duration of their productive careers. Awards are made on the basis of nationwide competition among candidates whose research has won the respect of scientific leaders in their fields.

2. Development Awards are made to enable institutions to finance positions for qualified scientists who have three years or more of relevant postdoctoral research or professional experience. These awards are made to individuals with a clear research potential and who have demonstrated a capacity for independent research but who require additional training and experience in a productive scientific environment in preparation for careers in independent research.

In 1962, a total of $208,363 was awarded for 16 Research Career Development Awards. These are located in 16 institutions in 12 States. No Research Career Awards were made in 1962.

THE ANNUAL REPORT OF THE SCIENTIFIC DIRECTOR

The National Institute of Dental Research

Calendar Year 1962

by

Dr. Seymour J. Kreshover

With the recent expansion of the Dental Institute's direct research operations, it is important that assessments of progress be made in the broad context of interpretation of mission and scope of responsibility.

Viewed in retrospect, the intramural program of the Institute has provided a significant element of leadership to the total national dental research effort. Currently and prospectively, this influence on research development in our universities and other nonfederal institutions gives evidence of further extension into the sphere of dental education. While earlier contributions to outside programs accrued as fringe benefits, there is today an increasing awareness by the Institute's scientists of a new role related to the broadening perimeters of their laboratories. In ever expanding degree, dental research is reaching into and complementing the allied biomedical, physical, and behavorial science fields.

Most responsible perhaps for this change has been the rapid and productive expansion of collaborative research ventures by Institute scientists and an increasing participation in the research and clinical associateship programs available at the National Institutes of Health for in-service training. In the conduct of these activities, the Dental Institute is well aware of its catalytic role in spurring the need for and the ultimate establishment of additional categorical dental research centers in university environments; each with the opportunity to influence further the shape of dental research, dental education, and dental practice.

More than a century ago, dentistry in this country ventured forth as an autonomous health profession, committed to a course of independence from medicine. With early educational emphasis on technical, material, and mechanical aspects of dental practice, it followed that the predominating research efforts in dental schools were channeled in parallel directions. It is not surprising, therefore, that the late-coming incorporation of the biological and medical sciences into dental educational curricula and allied research programs was more a grafting procedure than a naturally conceived part. The contrasting origin and development of the Dental Institute's research activity within a broadly conceived and multidisciplinary biomedical complex is perhaps the principal factor for its assumption of a pioneering role in the future of dental research and education in this country.

The diversity of activity represented in several recently initiated collaborative research programs is perhaps indicative of some trends, advances, and opportunities that lie ahead. As examples:

1. The Human Genetics Section, in collaboration with the University of Michigan, and under the sponsorship of the U. S. Atomic Energy Commission, the Rockefeller Foundation, and the Association for Crippled Children, is currently in its fourth year of study to evaluate the effects of inbreeding in a population where a large number of consanguineous matings had occurred and where detailed records of parents and offspring exist. Using facilities, of the Atomic Bomb Casualty Commission in Hiroshima and Nagasaki, Japan, and with the cooperation of the Japanese National Institute of Health, the team of investigators sought to determine the average numbers of deliterous genes in the selected populations, and assembled considerable data pertinent to stature, weight, and dental eruption. At present, the collaborating team member from the Dental Institute is on assignment to the Department of Genetics, University of Michigan, where he is completing data analysis.

2. In another collaborative study, the Human Genetics Section is participating with the National Institute of Neurological Diseases and Blindness in a contracted research program at the Clarke School for the Deaf in Northampton, Massachusetts, designed to analyze physical, biochemical, audiometric and clinical signs and symptoms incident to specific genetic types of hearing loss. The principal statistical method applicable to the analysis of assembled records and pedigree data will be by an electronic computer program designed by one of our staff for the study of discrimination and segregation patterns.

3. For the past five years, the Gnotobiotics Section, NIDR, has been engaged in a series of collaborative projects with Dr. Bengt Gustafsson of the Karolinska Institute in Stockholm, Sweden. The results of this association have been particularly fruitful in that it accomplished a partnership of competency in oral microbiology with a competency in nutrition and the development of germ free technics. For a period of six months during the current calendar year, the Chief of the Dental Institute's Gnotobiotic program was on assignment to Dr. Gustafsson's laboratory for the conduct of studies on the microbial aspects of dental and renal calculus formation in the rat. A report on this collaborative project will be forthcoming at an early date.

4. Following the demonstration by Dental Institute scientists that certain streptococci could serve as transmissible cariogenic agents in rodents previously free of caries, it became of importance to seek analogous situations in man. A group of hospitalized children at the National Children's Cardiac Hospital, Miami, Florida, and at Heart Haven Hospital, Lancaster, Pennsylvania, seemed to offer particularly suitable study material insofar as they had been maintained on an antibiotic regimen sufficient to depress any caries producing oral microflora. Accordingly, collaborative studies were negotiated which may provide the essential clinical follow-up information to the experimental lead that caries is a specific and transmissible bacterial disease. In parallel with these projects, a further collaborative study is being initiated with the Bureau of Indian Affairs to evaluate the cariostatic effect of a broad spectrum antibiotic of minimal toxicity (bacitracin) on selected groups of school children.

5. In the course of recent studies in the Dental Institute's Oral and Pharyngeal Development Section, it became apparent that currently available technics and devices for analysis of palatal impairments fail to distinguish normal valving from that found in disturbances of speech. Parallel observations by investigators at the State Institute for Speech Defectives in Copenhagen, Denmark, provided the basis for a contracted collaborative program to develop a device which would portray and measure air flow separately from nose and mouth during speech. Progress, to date, indicates that the new apparatus will provide accurate discrimination of speech valving at the palate in normal and abnormal subjects. It is anticipated that this contribution will make it possible to evaluate more effectively the benefits of prosthetic obturation and various forms of surgical repair in patients with partial and complete clefts.

6. During the past year, final field examinations were made in the 17 year study of the effects of fluoridation of community waters in Grand Rapids. This program, conducted by the Institute's Epidemiology and Biometry Branch in collaboration with the University of Michigan, the Michigan State Department of Health, and the City-County Health Department of Kent County, Michigan, not only established the practicability and efficacy of water fluoridation as a caries control measure, but also provided important evidence that optimal doses of fluoride, in the range of 1 ppm, caused minimal degrees of fluorosis (mottled enamel).

7. During the current calendar year, investigators from the Institute's Epidemiology and Biometry Branch collaborated with the Interdepartmental Committee on Nutrition for National Defense in surveys in Trinidad, Burma, Jordan, Bolivia, and the Blackfeet and Fort Belknap Indian Reservations of Montana. These studies continued to provide important information on patterns of oral disease prevalence and severity in various ethnic groups, and presented leads and challenges for future studies in depth. For example, it was found in Trinidad that unusually high prevalence of caries and periodontal disease co-existed in the same individuals. This is contrary to the usual finding in population groups of a significant dominance of one disease or the other, and raises some question regarding the validity of the long held view that dental caries and periodontal disease are mutually inhibiting.

The cited collaborative programs represent a rather limited number of intramural extensions made possible, in part, by contractual mechanisms. Proportionately, support for these activities is considerably less than the expenditures of other Institutes for their contracted collaborative programs which are, in large part, biometric, field and service oriented, and range from eleven per cent of their total direct operating budgets to more than four times the apportionment provided for intramural laboratory and clinical research at the National Institutes of Health.

A more representative portrait of the Dental Institute's breadth of activity and contribution to the broad field of biological and physical sciences may be provided by the extent of its less formal participation in joint research

enterprises with individual scientists and investigator teams of other categorical Institutes and outside institutions and laboratories. Based on information recorded in the annual project reports of the Dental Institute for the previous calendar year, it is estimated that 56 per cent of the professional staff engaged in active collaborative studies with one or more scientists from each of the other six categorical Institutes, as well as with a large number of other federal and nonfederal institutions. This compares most favorable with the estimated extent of similar, "between-scientists," collaborations participated in by the other six categorical research divisions of the NIH which, as recorded in last year's individual project reports, ranged from 32 to 48 per cent of the respective Institute's total complement of principal investigators. To a substantial degree, therefore, early concern has been dispelled on the matter of whether geographical consolidation of the Dental Institute's program under a single roof would contribute to an undesirable degree of segmentation and isolation of personnel and programs.

While the mechanisms for initiating collaborative research are often unpredictable, and the resulting program is the true measure of accomplishment, it is of some interest to note that, by a conservative estimate, at least 50 per cent of the Dental Institute's collaborations were consequent to other investigators seeking particular competencies among Dental Institute scientists. This fact alone provided our staff with an immeasurable sense of prideful participation in and contribution to the total mission of NIH as a unit structure as well as parent organization.

As we take note of today's major accomplishments in dental research, and speculate on the advances they might promise for tomorrow, the clarity with which we see the future is, of course, dependent on the distance in time that we try to bridge. It is evident, however, that unlike the frequent solo voyages of the past, our journey into tomorrow will be with a multidisciplinary crew competent to deal with the complexities of modern research navigation. While speculation on matters of research can be a most hazardous undertaking, the more evident events on the horizon would seem to be related to the impact of dental research itself on the teaching and practice of dentistry.

Following the discovery some ten years ago, that dental caries cannot occur in the absence of bacteria, further successes were gained by the isolation of decay producing organisms, and the establishment of a direct cause and effect relationship between the disease and a particular oral flora peculiar to the animal species under study. While the evidence of such highly specific host-parasite relationship served to emphasize the magnitude of the problem still faced in attempting to identify a similar causative microbial factor in man, an already considered objective is to develop a method of caries prevention by specifically acting anti-bacterial agents or related means. Leaving the dental practitioner manpower problem of the future to others more qualified to anticipate and estimate needs, one can be sufficiently objective to forecast the possibility of prophylactic procedures for prevention of dental caries becoming a matter so routine as to be handled by pediatrician and dentist alike.

In parallel with this expected achievement in the prevention and control of dental caries, there emerges the interesting speculation that as more teeth

are saved from caries, more susceptible tissue sites may be preserved for attack by periodontal disease. How realistic a forecast is this for the dental practitioner of 1970 or 1980? Since formation of dental calculus in the gingival area is recognized as perhaps the most immediate exciting factor in periodontal disease, systematic studies of calculus are essential. One such investigation has shown that various species of streptococci outnumber all other types of microorganisms at all stages of calculus formation. This, we recall, is contrary to the long held view that filamentous forms predominate, and may present to some a possibility that adequate measures can be found to prevent ectopic calcifications by the elimination of suspected causative bacteria. Other studies are seemingly pointing to comparable relationships between specific microorganisms and certain types of periodontal disease. Thus, the prospect is for the development of prophylactic measures against periodontal disease similar to those envisioned for the prevention of dental caries.

With parallel advances expected in other categories of oral disease, the bridge that has long separated the basic from the clinical fields of dentistry promises to become a well travelled road of personnel exchange and communications. Similarly, there is every assurance of a further narrowing of the still existing gap between dentistry and its allied health professions. Thus, in the definition of and approach to long range program goals, principally through the effective utilization of scientific manpower, the Dental Institute has continued to follow a practice of formally established collaborative and integrated planning among its program leaders. Quite naturally, this undertaking requires an appraisal of current programs in order to establish a baseline upon which to make reasonable assumptions of future trends and to construct and reconstruct along lines of promising productivity. In this responsibility, the Institute's scientific leaders have received an important measure of support in the expressions of confidence by the Board of Scientific Counselors.

Summary statements by the Dental Institute's program leaders, introductory to the project reports of their principal investigators, provide a highlight account of research activities by organizational categories.

NATIONAL INSTITUTE OF DENTAL RESEARCH

Individual Project Reports
Calendar Year 1962

LABORATORY OF MICROBIOLOGY:

1. R. J. Fitzgerald: The Use of Germfree and Gnotobiotic Animals in the Study of Problems Relating to Oral Disease

2. B. Hampar: The Mechanism of Cell Transformation Induced by an Animal Virus

3. E. G. Hampp: Studies on the Oral Spirochetes

4. A. Howell, Jr.: A Study of Filamentous Organisms Isolated from the Oral Cavity of Man and Animals and Their Relationship to Diseases of the Oral Cavity

5. A. Howell, Jr.: A Study of the Genus Actinomyces

6. H. V. Jordan: Metabolism of Oral Microorganisms

7. M. I. Krichevsky: The Biochemistry of the Differentiating Cellular Slime Mold, Dictyostelium discoideum

8. R. H. Larson: Role of Genetic, Microbiological, Nutritional and Host Factors on Experimental Dental Caries

9. S. E. Mergenhagen: Mechanisms of Action of Oral Bacteria and Their Products in Oral and Systemic Disease

10. A. L. Notkins: The Relationship Between Tissue Enzymes and Experimental Infection

11. A. A. Rizzo: The Role of Bacteria in the Formation of Dental Calculus

12. A. A. Rizzo: Experimental Ulcer Formation in Oral Mucosa

13. M. Rogosa: Immunological, Biochemical, and Pathological Factors in the Taxonomy and Ecology of Oral Bacteria

14. H. W. Scherp: Manifest and Inapparent Viral Infections of Oral Tissues

15. T. Shiota: Nutrition and Metabolism of Lactic Acid Bacteria

16. C. L. Wittenberger: Studies on Lactate Fermentation by Butyribacterium rettgeri

Annual Report of the Laboratory of Microbiology
National Institute of Dental Research
Summary Statement

During 1962, the research programs of the Laboratory of Microbiology continued to fall quite naturally into a long-range pattern encompassing the categories of periodontal disease, dental caries, microbial taxonomy, microbial physiology, immunology, and virology. The work of each individual investigator, however, extends into several areas, as it should in a healthy research program.

From the viewpoint of direct relevance to oral disease, the most notable achievement of the year was the first fruition of a collaborative investigation with the Laboratory of Histology and Pathology, which demonstrated for the first time the transmissible infectious nature of a form of periodontal disease in hamsters and identified a specific type of filamentous microorganism as an essential etiologic factor. The availability of this model system opens the way for controlled study of the undoubtedly multiple factors, nonmicrobial as well as microbial, that determine the course of periodontal disease in general. Since in many ways this experimental syndrome resembles chronic periodontitis in man, further study of its pathogenesis should help elucidate the nature of this major human disease. Already, the ecologic subtlety of the experimental disease has been revealed by the fact that the essential etiologic microorganism can be established readily in germfree mice but that it produces no periodontal pathosis in them. These observations may indicate the necessity for species-specific host factors; on the other hand, they may indicate the necessary participation of additional microbial species.

Similar collaborative studies established previously the transmissible infectious nature of experimental dental caries in hamsters and rats and identified a particular kind of streptococcus as essential microbial factor. These studies have been recognized repeatedly by awards, culminating in 1962 with the Joachim International Research Prize of the International Dental Federation, which has been awarded only once every several years and is the subject of keen international competition. Continuation of these studies emphasizes the importance of quite sophisticated correlated investigations in microbial physiology; that is, to date no characteristics have been found to explain why certain strains of streptococci are cariogenic in hamsters and rats, whereas other strains, seemingly the same by a variety of criteria, induce no caries. Such investigation should be greatly facilitated by development during this year of a simplified, chemically defined culture medium for these streptococci, which makes it possible to study them under closely controlled conditions.

Intimately influencing the action of microbial agents of caries are genetic and nutritional determinants; however, we know now that in past studies the significance of the latter was obscured by doubt whether the experimental animals harbored the essential cariogenic flora. Experiments in this Laboratory have now confirmed the inherent genetic resistance of certain strains of rats to experimental caries, even when they are adequately exposed to a cariogenic oral flora and are maintained on a diet conducive to caries. As expected,

the progeny from mating such rats with a caries-susceptible strain showed intermediate degrees of susceptibility. Past reports have stated that maintenance of pregnant rats on a high-sugar diet resulted in increased susceptibility to dental caries in the offspring; however, it has now been shown in this Laboratory that when measures were taken to equalize the oral flora, the progeny of mothers maintained on a low-sugar diet developed just as much caries as the others. The apparently greater susceptibility of the "high-sugar" group evidently resulted from transmission of an intensified cariogenic flora from the mothers to the offspring.

The accumulation of dental calculus, with its accompanying microbial components, is recognized as the most important immediate provocant of periodontal disease. Previous observations in this Laboratory indicated that, contrary to the general impression, filamentous forms did not bear a specific etiologic relation to calculus formation; a wide variety of other oral microorganisms predominated in all stages of developing human calculus, and calcified equally well in a model system in vivo, namely, within cellophane sacs implanted intra-abdominally in rats. The latter system has now been extended to include calculus deposition on sterilized teeth within the sacs. Under these conditions, present results indicate that filamentous bacteria may play a rather specific role, e.g., deposition occurred in the presence of Actinomyces israelii, but not an oral diphtheroid. On the other hand, our gnotobiotic studies have shown that calculus accumulates about the teeth of germfree animals and has a microstructure very similar to that of ordinary calculus, except for the substitution of an amorphous organic matrix for bacteria. Evidently the tendency to form calculus is inherent in the host; the critical determinant is a suitable organic matrix upon which mineralization may occur, and bacteria constitute a particularly effective one. This conclusion is substantiated by other experiments showing that calculus deposition in ordinary germ-bearing animals is invariably more extensive than in comparably treated germfree animals; that it is not much affected by a variety of chemical, nutritive, and physical alterations of the diet; but that it is strikingly reduced by inclusion of a minute amount of an antibiotic, erythromycin, in the diet.

Our attempts to characterize specific cariogenic streptococci from rats and hamsters, and the filamentous form essential for periodontal pathosis in hamsters, have emphasized anew the continuing great importance of classic systematic study of the oral flora, which has lagged behind that of most other parts of the animal and human body. Without a large reservoir of such fundamental information, we are seriously limited in our understanding of the ecological and pathological potentialities of these organisms, and different investigators can not be certain whether they are examining the same organism. Thus, the streptococci and the filamentous organism just mentioned seem to belong to previously undescribed groups, and will require further analysis. During this year, it has become possible to delineate a much more accurate cultural, biochemical, and serological characterization of the genus Veillonella, the second most numerous bacteria in the human mouth. Particularly significant is serological analysis of the toxic lipopolysaccharide antigens of the cell surface, because it reveals hitherto unsuspected sharp distinctions between the strains of Veillonella from different individuals. Similar analysis has revealed equally numerous and specific serogroups in the fusobacteria, which

have long been implicated in periodontal disease. Also during this year, criteria for the genus Actinomyces were reviewed. It was concluded that the characteristics of these organisms had been stable on prolonged cultivation; and that the species of this genus must be differentiated by morphological criteria and comparative rates of growth, for the usual physiological and biochemical tests were of no value. Finally, an important contribution was the development of a simplified improved culture medium for oral and other spirochetes, which should greatly facilitate their isolation and mass cultivation.

In the field of microbial physiology, unusual metabolic functions of the vitamin, lipoic acid in Butyribacterium rettgeri were elucidated further. Though essential for growth on lactate media, lipoic acid does not participate in pyruvate fermentation by this organism, and is not required for its growth on glucose media; however, lactate is a major product on the latter media. Extracts of B. rettgeri grown on glucose contain a DPN-linked lactic dehydrogenase, lacking in cells grown on lactate. Extracts of the latter, however, contain an enzyme system capable of coupling anaerobic dissimilation of lactate to the reduction of ferricyanide. It is believed that the DPN-linked lactic dehydrogenase functions primarily in formation of lactate from pyruvate, whereas the ferricyanide reaction reflects an enzyme system primarily involved in dissimilation of lactate. Since end products are the same, whether from glucose without lipoic acid or from lactate with lipoic acid, the latter seems to function primarily in the electron transport system involved in disposition of lactate hydrogens. In another study, continued investigation of the biochemical factors initiating and controlling morphogenesis of Dictyostelium discoideum showed that the amino acid, histidine, stimulated morphogenesis at pH 5 but not at pH 7. Since equal amounts of histidine were taken into the cells at these pH values, its action seemed not to depend on intracellular accumulation but to be external. Histidine decreased the uptake of exogenously supplied amino acids into the internal free pool; it decreased the leakage of ultraviolet-absorbing material from the cells but not the leakage of protein. A direct correlation was found between the ability of materials to stimulate morphogenesis and their ability to prevent leakage of ultraviolet-absorbing material from the cells. Another project continued to study enzymatic synthesis of the vitamin, folic acid, by lactobacilli; from data obtained by electrometric titration, acid hydrolysis, enzymatic hydrolysis, and spectrophotometry, it was concluded that enzymatic coupling brings together 2-amino-4-hydroxy-6-pteridylmethyl-pyrophosphate and para-aminobenzoylglutamate to form dihydrofolic acid and free pyrophosphate.

Immunologic investigations emphasized the immunochemical analysis of endotoxic lipopolysaccharide antigens of oral bacteria and the alteration of nonspecific host resistance by these substances. Lipopolysaccharides from species of Veillonella, Fusobacterium, Leptotrichia, Selenomonas, and Neisseria were characterized as to molecular weight (greater than 50,000 and polydisperse), saccharide units (glucosamine, galactosamine, methylglucose-like, sialic-acid-like), and lipid components (cephalin, plasmalogen, fatty acids). Although the isolated lipopolysaccharides did not engender antibodies when injected parenterally into rabbits, they sensitized erythrocytes to agglutination by homologous antibody and formed specific precipitates with antibodies. By a

combination of agglutination and antibody-absorption techniques, the lipopolysaccharides were shown to be part of the mosaic of surface antigens of their parent bacteria. By indirect hemagglutination and its inhibition by solutions of the lipopolysaccharides it was possible to delineate clear-cut serogroups of strains of Veillonella, Fusobacterium, and Leptotrichia, which should assist studies of the distribution of these organisms. In other experiments, it was shown that prior treatment of mice with vaccines of oral gram-negative bacteria, or with minute doses of their lipopolysaccharide endotoxins, effects a biphasic, nonspecific change of their susceptibility to experimental bacteremia with oral viridans streptococci, to which they are ordinarily quite resistant. During the first 12 hours, susceptibility is increased tenfold; after 24 hours, resistance is greater than normal. The latter effect depends not only on an increased blood clearance by the reticulo-endothelial system but also on increased phagocytic capacity of mononuclear and polymorphonuclear leucocytes in the treated animals. This state seems to be attributable at least in part to the nontoxic lipid moiety of the endotoxin, which is released by enzymes of the liver; that is, markedly increased nonspecific resistance to infection can be induced without a lag period by injection of minute amounts of the partially purified lipid, released in vitro by either acid hydrolysis or digestion with liver homogenate. Another aspect of the biology of endotoxins has been the question whether animals become more reactive to them following exposure to an endotoxin or to the bacteria producing it. We have now answered this question affirmatively by showing that mice bearing the usual coliform flora of their species are much more susceptible to an E. coli endotoxin than are their germ-free or coliform-free counterparts.

In virology, our continued serological analysis of herpes simplex virus has shown in a number of cases that a different strain is present in each successive recurrence of fever blister in the same individual. This observation throws a new light on the natural history of this common infection, which usually has been attributed to exacerbations of the identical virus. Other experiments have now succeeded in producing primary herpetic ulcerations in the oral mucosa of rabbits, thus providing a useful model system for study of immunity, exacerbation, and therapy of such infections. In another direction, investigation was started on the mechanisms and consequences of animal cell transformation, with chromosomal aberrations, induced by herpes simplex virus. Investigation continued on the virus designated as lactic dehydrogenase (LDH) agent, which was discovered as a contaminant of a number of transmissible tumors of mice, in which it resulted in a five- to tenfold elevation of the plasma LDH. Now it has been shown to produce elevations also of the plasma isocitric dehydrogenase, malic dehydrogenase, phosphohexose, isomerase, and glutamate-oxalacetate transaminase, but not of alkaline phosphatase or aldolase. The well known elevation of plasma enzymes that accompanies development of transmissible tumors has been shown to be truly attributable in part to the tumors, but infection with the LDH agent superimposes an additional increase. This virus is characterized by several unusual properties, including rapid initiation of multiplication within a few hours after infection, associated with extraordinarily high titers of virus in plasma within 24 hours; viremia persisting for at least a year, suggesting a poor antibody response; similarly prolonged elevation of a number of plasma enzymes, evidencing some sort of cellular alteration; and absence of discernible illness and gross or histological

evidence of pathologic change. Spontaneous transmission of the LDH agent from infected to uninfected mice in the same cage is infrequent; the virus has been recovered from saliva, feces, and urine only early in the course of infection. The properties of this agent make it an uncommonly promising subject for investigating virus-host cell interactions.

Serial No. NIDR-1
1. Microbiology
2. Gnotobiotics
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1962

Part A

Project Title: The Use of Germfree and Gnotobiotic Animals in the Study of Problems Relating to Oral Disease

Principal Investigator: Dr. R. J. Fitzgerald

Other Investigators: Dr. H. V. Jordan
Dr. P. H. Keyes
Dr. J. Theilade
Dr. Bo Krasse

Cooperating Units: DRS-LAB Germfree Animal Production Unit.
Dr. Carl Miller

Man Years (calendar year 1962):

Total:	5-1/2
Professional:	1-1/4
Other:	4-1/4

Project Description:

Objectives:

A continuation of Project NIDR-1 (1961) in which germfree and gnotobiotic animals are used in the study of dental caries, calculus formation and periodontal disease.

Methods Employed:

Germfree animals or animals known to be lacking certain types of microorganisms are inoculated with pure cultures of specific microorganisms in order to determine the relative importance of these agents in the etiology of oral disease.

Major Findings:

1. In 1962, considerable effort was devoted to establishing the inbred NIH strain of Fischer rats in the germfree state. It was anticipated that this strain would be of great usefulness for gnotobiotic studies because it was genetically more homogeneous

than the Lobund strain and would be expected to respond in a more uniform and reproducible manner to experimental challenges of various types. While these animals proved to be excellent in regards to breeding performance and ease of handling, their response to cariogenic challenge was disappointing. It was found that the molar teeth of this strain underwent rather severe attribution on the occlusal surfaces on the coarse particle cariogenic diets employed. As a result the pits and fissures normally subject to caries attack were worn smooth before caries could become extensive. For this reason the germfree colony of the NIH Sprague-Dawley rats is now being established. This latter strain has been used in the majority of our studies in conventional animals and has been uniformly susceptible to caries on the coarse particle diet employed even after it has been sterilized by autoclaving.

2. In collaboration with the Laboratory of Histology and Pathology, NIDR, histological and electron microscope studies were performed on dental calculus from germfree and conventional animals. Preliminary results indicate that the microstructure of calculus from germfree animals is very closely analogous to conventional calculus except for the occurrence in the latter of considerable numbers of microorganisms. The organisms seem to provide an additional source of "matrix" upon which mineralization may occur. In contrast the matrix remaining after decalcification of "germfree" calculus appeared to be amorphous and uniform.

3. In additional studies using conventional rats variations in the particle size and fat content of diets known to produce calculus were investigated. Such modifications of the diet had a relatively small effect on the degree and location of the calculus deposits when compared with the effect of an antibiotic, erythromycin, which was incorporated in the food at a level of 50 μg per gram of diet. Regardless of the type of diet the presence of the antibiotic resulted in a marked diminution of calculus.

4. Attempts to protect hamsters and rats against dental caries by immunization with specific cariogenic streptococci have continued to yield negative results. During the past year several routes of administration of the vaccine have been tested, along with various antigen preparations. It has been found that intraperitoneal injection of rats or hamsters with the vaccine in Freund's adjuvant will result in high agglutinating titres in the ascites which accumulates but that sera from these same animals showed only very slight or even no agglutinin titres.

Significance to Dental Research:

The calculus studies reported supplement and extend previous observations on this subject and support two conclusions which may at first glance appear contradictory but which in fact are quite compatible as an explanation of the condition as it occurs in nature, i.e., in the presence of a conventional microbial environment.

The first point is that dental calculus may occur on a number of different diets of varying composition both in regard to chemical, nutritive, and physical characteristics. It may also occur in the absence of bacteria. Thus it appears that the tendency to calculus formation is something that is inherent in the host and to a certain extent independent of external influence.

However, calculus deposits are invariably more extensive in conventional animals than in comparably treated germfree animals, and at least one antibiotic, erythromycin, has been shown to reduce to a marked degree the severity of the deposits in conventional animals. This is interpreted to mean that the presence of certain microorganisms (or their metabolic products) on the teeth or gingivae can have a profound influence on the severity of calculus deposition. The fact that these organisms are susceptible to erythromycin reduces to some degree the task of isolating and identifying them although the list of possible candidates is still quite extensive.

Proposed Course of Project:

The future course of this project will be along the lines already established. The approach is flexible enough to exploit provocative leads as they arise. Other examples of the usefulness of the gnotobiotic animal to dental research can be found in the reports of Drs. Jordan, Mergenhagen, and Jensen of the Laboratory of Microbiology and Dr. Keyes of the Laboratory of Histology and Pathology.

Part B included Yes

Serial No. NIDR-1
1. Microbiology
2. Gnotobiotics
3. Bethesda, Md.

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Individual Project Report
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Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Jordan, H. V., Fitzgerald, R. J., and Berger, N. D. A Comparison of the Caries Inhibitory Potential of Sodium Metabisulfite and Related Compounds. J. Dental Res. 41: 61-64, 1962.

Keyes, P. H., and Fitzgerald, R. J. Dental Caries in the Syrian Hamster. IX. Arch Oral Biol. 7: 267-278, 1962.

Keyes, P. H., Fitzgerald, R. J., Jordan, H. V., and White, C. L. The Effects of Various Drugs on Caries and Peridontal Disease in Albino Hamsters. Arch. Oral Biol. (Special Supplement) 7: 123-141, 1962.

Gustafsson, B. E., Daft, F. S., McDaniel, E. G., Smith, J. C., and Fitzgerald, R. J. Effects of Vitamin K-Active Compounds and Intestinal Microorganisms in Vitamin K Deficient Germfree Rats. J. Nutrition 78: 1962 (in press).

Larson, R. H., Zipkin, I., and Fitzgerald, R. J. Effect of Dehydroacetic Acid and Tetracycline on Caries Activity and Its Transmission in the Rat. J. Dental Res. (accepted for publication).

Honors and Awards relating to this project:

Joachim International Research Prize (In collaboration with Paul H. Keyes), International Dental Federation, Cologne, W. Germany July 1962.

First Prize Scientific Exhibit, Annual Meeting American Dental Association, Miami, Florida, November 1962.

Serial No. NIDR-2
1. Microbiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1962

Part A

Project Title: The Mechanism of Cell Transformation Induced by an Animal Virus

Principal Investigator: Dr. B. Hampar

Other Investigators: None

Cooperating Units: University of Buffalo, Buffalo, New York
Children's Cancer Research Foundation, Boston, Massachusetts

Man Years (calendar year 1962):

Total:	3/4
Professional:	1/2
Other:	1/4

Project Description:

Objectives:

1. To determine the mechanism of herpes simplex virus-induced transformation of Chinese hamster cells in vitro.

2. To compare the sites of action of virus on chromosomes with those of other mutagenic agents.

3. To determine possible mutagenic action of virus on cells at the antigenic level.

Methods Employed:

Cells grown in culture are infected under specific conditions with herpes simplex virus. Cell survival is measured by plating efficiency studies. The kinetics of cell killing with respect to time of killing is studied and various methods for delaying or inhibiting certain cell processes are employed to determine their effect on virus-induced killing.

Part A (continued)

The chromosomes of infected cells are studied at various times after infection and the types and location of chromosomal aberrations and/or alterations are determined. These sites on various chromosomes are compared morphologically with sites known to be affected by other mutagenic agents. Attempts are being made to induce tolerance in animals to control cells and infected cells with the aim of determining possible antigenic changes in infected cells.

Major Findings:

1. Cell killing by herpes simplex virus occurs only following shaking of cells with virus. Chromosomal aberrations occur during the first post-infection division. Kinetic studies indicate that killing occurs in these cells which divide first, even though practically all cells are susceptible to killing when high multiplicities of infection are used. Since virus production is small, cell killing appears to result from physiologic alterations in the cell rather than a specific effect of virus as seen in other virulent systems. Survival of cells which divide late may imply a repair type mechanism.

2. Chromosomal alterations are seen in clonal isolates of infected cells. The location of these alterations coincides with the sites affected by 5-bromodroxyurdine, hydroxylamine, and X-irradiation.

Significance to Dental Research:

An understanding of the mechanism of action of herpes virus in vitro resulting in cellular alterations may also apply to clinical herpetic lesions seen in and around the oral cavity. A relationship between such changes and recurrent aphthous lesions may also exist.

Proposed Course of Project:

Present studies will be extended using diploid lines of Chinese hamster cells to better localize the chromosomal lesions. The kinetics of cell killing following shaking of cells with virus and further criteria of cellular alterations will be explored.

Part B included No

Serial No. NIDR-3
1. Microbiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1962

Part A

Project Title: Studies on the Oral Spirochetes

Principal Investigator: Dr. E. G. Hampp

Other Investigators: None

Cooperating Units: American Dental Association

Man Years (calendar year 1962):

Total:	1
Professional:	1
Other:	0

Project Description:

Objectives:

This is a continuation of Project NIDR-7 (1961), with the following immediate objectives:

1. The development of an easily prepared reproducible liquid medium from dehydrated products and superior to the modified Huntoon's broth for facilitating the mass cultivation of the smaller oral treponemes and Borrelia vincentii.

2. Growth of the various strains of Borrelia buccalis in an agar-free medium, which has offered difficulties in past studies.

3. Application of new experimental broth media for the mass cultivation of five of the "cultured strains" of Treponema pallidum. Collection of these organisms to evaluate their endotoxic activity, which has not been reported in the literature.

Methods Employed:

1. The complex modified Huntoon's broth has been used in the past to obtain cultures of the small oral treponemes and B. vincentii. However, their growth is minimal in this medium as compared with other oral isolates grown in their respective media. Such studies are essential to further investigations on the chemical properties

and biological activity of the spirochetal endotoxins and on the physiology and serology of these organisms. It was previously reported that a two-step method was studied in an effort to simplify and to avoid precipitates in the preparation of the complex Huntoon's medium, while maintaining the growth-promoting qualities of the medium. However, these efforts were not very successful. This year, as an outgrowth of previous studies, various dehydrated products ordinarily used for bacteriologic media were investigated to develop an easily reproducible clear medium for our purposes. This included all the peptones produced by Baltimore Biological Laboratory and Difco, and certain ones of Oxoid. Yeast extract from various suppliers, supplements of yeast extract, serums, fatty acids and additives of various types were included.

2. It was reported last year that one out of four strains of B. buccalis was successfully grown for the first time in broth medium. This was accomplished by cultivation of the organisms in diminishing amounts of agar contained in a fluid medium over an extended period of time. By the process of selection or adaptation the organisms were induced to grow free of agar. This strain of organisms (GT2) was also used along with the small oral treponemes and B. vincentii to evaluate the growth potential of new broth medium preparations for possible mass culture of this spirochete for other studies.

3. The "cultured strains" of T. pallidum, which include the English Reiter, Kazan, Nichols, Noguchi and Reiter treponemes, were also used along with the oral spirochetes to evaluate the growth potential of the various broth media prepared from dehydrated products. These results were also compared with growth of the same organisms in Huntoon's broth and Spirolate Broth (BBL). The latter medium has been advocated for growth of the Reiter treponeme. In addition, cells of all such strains are being collected for studies on endotoxic activity (which thus far has not been reported in the literature).

Major Findings:

1. A reproducible, easily prepared crystal clear broth medium has been developed from dehydrated products which is superior to Huntoon's broth for mass cultivation of the oral spirochetes. The latter medium requires about one week to prepare, whereas the new broth may be made in 30 minutes, depending on the volume required. It withstands sterilization by autoclaving instead of the time-consuming fractional process previously used and is inexpensive to prepare. It is composed of thiotone (BBL), yeast extract (BBL), glucose, sodium chloride and water. A veal heart or other meat base is not required. Individual substitution of all other commercial peptones (BBL, Difco, certain Oxoid products) was found to be inferior to thiotone even though certain of the peptones supported spirochetal growth. Yeast extract (BBL) was found to be superior to all other types of the same material. Using optical density and cell

enumeration for the evaluation of cell crops, it was found that the growth of the small oral treponemes was tripled and that of B. vincentii was doubled and in some instances almost tripled as compared with the same organisms grown in Huntoon's broth. Gentle agitation of one-liter cultures of these organisms with magnetic stirring equipment slightly increased the yield of the spirochetes. In addition, the broth may be used to prepare an excellent solid medium by the addition of 0.8% agar. It will support the growth of the small oral treponemes and B. vincentii; however, only 3 out of 4 cultures of B. buccalis survived in this agar medium. Studies are in progress on this phase of the problem since a simplified and easily reproducible agar medium is needed both for isolation and cultivation of oral spirochetes.

2. The new basic broth medium has been used to cultivate 3 out of 4 strains of B. buccalis. The organisms do not require adaptation or selection over a period of time but may be transferred directly from their agar maintenance medium to the broth. Further, cell crops of B. buccalis (GT2) are tripled in amount as compared with the same strain grown in Huntoon's broth. The other two strains (NH-1 and P.B.B.) grow equally as well as GT2 in the new broth medium. Thus far, the latter two strains of spirochetes have not been grown in Huntoon's broth. Therefore, comparisons could not be made between the two media.

3. The five "cultured strains" of T. pallidum grew as well in the new broth medium as in Huntoon's broth and better than in Spirolate Broth (BBL), which is advocated for the Reiter treponeme. These organisms are being mass cultured and stored until sufficient amounts of cells are obtained to study endotoxic activity. It is suspected that they contain endotoxins; however, there are no reports in the literature on this subject.

Significance to Dental Research:

1. The development of an easily prepared and reproducible medium for increasing cell crops of oral spirochetes is essential for preparing endotoxins for further study of their chemical composition, biologic activity, physiology and serology.

2. Although not of dental significance, the work with the "cultured strains" of T. pallidum would be of value in the general field of treponematoses, where the same problems are encountered in the study of various other spirochetes of significance to man, particularly T. pallidum.

3. The success obtained in preparing a satisfactory solid agar medium from the new broth base should simplify isolation and cultivation of oral spirochetes and, therefore, encourage other

investigators to study these organisms and their relationship to oral diseases.

4. For the first time, B. buccalis can be grown in a broth medium on the first transfer from the agar maintenance medium without prolonged adaptation from an agar-containing medium. Thus, this organism can be further studied in relation to oral diseases.

Proposed Course of Project:

These studies will be continued and expanded so that future experiments may be designed to elucidate the etiology of ulcerative gingivostomatitis, in which the oral spirochetes have long been implicated.

Part B included Yes

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Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Hampp, E. G., and Mergenhagen, S. E. Experimental Intracutaneous Fusobacterial and Fusospirochetal Infections. J. Infect. Dis., Jan. Feb. issue, 1963 (in press).

Honors and awards relating to this project:

In 1962, an alumni citation from Washington University, St. Louis, Missouri. "In recognition of his service to the science of dentistry through teaching and through significant research in the field of microbiology."

Serial No. NIDR-4
1. Microbiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1962

Part A

Project Title: A Study of Filamentous Organisms Isolated from the Oral Cavity of Man and Animals and Their Relationship to Diseases of the Oral Cavity.

Principal Investigator: Dr. Arden Howell, Jr.

Other Investigators: Dr. H. V. Jordan and Dr. Paul Keyes

Cooperating Units: None

Man Years (calendar year 1962):

Total:	1-3/4
Professional:	3/4
Other:	1

Project Description:

Objectives:

To study the incidence and identity of filamentous organisms in the oral cavity of man and certain animals, and to determine their significance, if any, to oral disease. This is a continuation of Project NIDR-5 (1961).

Methods Employed:

Standard bacteriological procedures, modified as necessary for the isolation and identification of filamentous organisms.

Major Findings:

It was previously reported (1959, 1960) that a nocardia-like organism had been isolated from the oral cavity of several varieties of hamsters. To date over 150 strains of this organism have been isolated from 30 of 48 hamsters sampled and they have been found to be associated with the development of periodontal disease in these animals. In fact, this organism has not been isolated from any animal which did not show some degree of periodontal disease, and it is usually the predominant organism isolated from animals with extensive periodontal disease but

Part A (continued)

without caries. Detailed studies of the morphological, physiological, and biochemical characteristics of a considerable number of strains of this organism indicates that they form a homogeneous group and represent a distinct species. Comparisons have been made with several strains of Nocardia asteroides (the type species of Nocardia) and Nocardia salivae, which was isolated from the oral cavity of humans and described recently by Davis and Freer. It appears, from studies to date, that the organisms from the hamster are sufficiently related to N. salivae to be included in the same genus, but that both organisms are so different, physiologically and biochemically, from N. asteroides that neither can be considered as a species of Nocardia or any other recognized genus of the family Actinomycetaceae, in which group all three organisms must be placed. For example, typical colonies of N. asteroides, at least on certain media, are tough and leathery with an abundant aerial mycelium; growth is very slow. In a well buffered broth containing glucose, strains of N. asteroides form a tough pellicle, leaving the medium clear; in such a broth, no acid is produced within 30 days. In addition, N. asteroides grows well on a medium containing inorganic salts as the sole source of nitrogen. In contrast, both of the other organisms form colonies which are usually soft, creamy, or, in the case of the organism from the hamster, mucoid, without aerial mycelium; growth of both is very rapid; in the same well buffered broth containing glucose, mentioned above, both produce a diffuse, granular, or mucoid type of growth, and a considerable quantity of acid (mostly lactic) within 3-4 days; neither will grow on a medium containing inorganic salts as a sole source of nitrogen.

N. salivae and the organism from the hamster differ in micro - and macro-colony formation, although both are essentially aerobes and catalase positive. The former (N. salivae) does not require CO_2 for growth, whereas all strains of the latter do, except in the presence of whole blood. N. salivae liquefies gelatin but does not hydrolyze starch, whereas the organism from the hamster does the reverse. Other specific differences, such as fermentation of specific carbohydrates, action on nitrate, etc., are also evident in the physiological activity of these two organisms.

Significance to Dental Research:

From recent preliminary in vivo studies by Drs. Keyes and Jordan (NIDR) it appears that the presence of this filamentous organism, isolated from periodontal plaques in hamsters, is necessary for the development of experimental periodontal disease in these animals. Therefore, knowledge of its physiological and biochemical activities, and its relationship to other similar filamentous

Part A (continued)

organisms, appears to be most important.

Proposed Course of Project:

1. To complete the comparison of the filamentous organism isolated from the hamster with that described as Nocardia salivae and with N. asteroides.

2. To attempt to solve, in cooperation with Dr. Lucille Georg, Chief, Mycology Unit, CDC, PHS, the proper nomenclature and taxonomy of N. salivae, the organism isolated from the periodontal plaque of hamsters, and possibly similar organisms previously described in the literature.

Part B included: Yes

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Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Howell, A. Jr., Stephan, R. M., and Paul, F. Prevalence of Actinomyces israelii, A. naeslundii, Bacterionema matruchotii, and Candida albicans in Selected Areas of the Oral Cavity and Saliva. J. Dent. Research 41: 1050-1059, 1962.

Honors and Awards relating to this project:

None

Serial No. NIDR-5
1. Microbiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1962

Part A

Project Title: A Study of the Genus Actinomyces

Principal Investigator: Dr. Arden Howell, Jr.

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1962):

Total:	1/2
Professional:	1/4
Other:	1/4

Project Description:

Objectives:

To study the morphological and physiological characteristics of specified strains of organisms described by various investigators as A. israelii, A. bovis, and A. naeslundii as a basis for a round-table discussion on the genus held at the 8th International Congress for Microbiology, Montreal, Canada, August 19-26, 1962. This study, and participation in the Round Table, was undertaken at the request of Dr. John Slack, Chairman, Department of Microbiology, West Virginia University Medical Center.

Methods Employed:

Standard bacteriological procedures, modified as necessary for the organisms under study, reported in detail in previous publications (Howell, A., Jr., and Pine, L. : J. Bacteriol. 71: 47-53, 1956; Pine, L., and Howell, A., Jr.: J. Gen. Microbiol. 15: 428-445, 1956; Pine, L., Howell, A., Jr., and Watson, S. J.: J. Gen. Microbiol. 23: 403-424, 1960) were employed throughout the study.

Major Findings:

The strains of Actinomyces supplied by Dr. Slack were studied in detail, both morphologically and physiologically. They were compared simultaneously with cultures of the same strains which

have been maintained by the principal investigator in this laboratory for some time and were previously described in the above listed publications and in J. Bacteriol. 78: 82-95, 1959. In general, no significant differences were found in the two sets of cultures nor in results previously reported for these strains. It was again concluded that all three of these species of Actinomyces conform to the description of the genus as given in the 7th Edition of Bergey's Manual of Determinative Bacteriology and are catalase negative. Furthermore, species of this genus must be differentiated on the basis of (1) their morphology on a standard medium such as brain heart infusion agar (Difco Laboratories), with particular reference to their micro- and macro-colony formation and (2) their relative rates of growth. Physiological and biochemical tests are of no value for this purpose. The latter conclusion was further supported by an extensive review of the literature in which the physiological and biochemical characteristics of the following were tabulated: (1) 283 strains described as A. israelii isolated from human or animal lesions; (2) 158 strains of the same species isolated from the oral cavity of humans; (3) 51 strains of A. naeslundii isolated from the oral cavity of humans; and (4) 180 strains morphologically typical of A. bovis, isolated from animals.

Significance to Dental Research:

Proper identification of species of Actinomyces, isolated from the oral cavity of man, is most important, in view of their prevalence in the oral cavity and possible role in the production of caries and peridontal disease (reported elsewhere).

Proposed Course of Project:

As a result of the Round Table held at Montreal it is possible that a Subcommittee on Taxonomy and Nomenclature of the Microaerophilic Actinomycetes will be formed to further study this group of organisms. If such a committee is formed, I have been invited to be one of the members

Part B included No

Serial No. NIDR-6
1. Microbiology
2. Gnotobiotics
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1962

Part A

Project Title: Metabolism of Oral Microorganisms

Principal Investigators: Dr. H. V. Jordan

Other Investigators: Dr. R. J. Fitzgerald
Dr. P. H. Keyes
Dr. S. A. Narrod

Cooperating Units: None

Man Years (calendar year 1962):

Total:	2-1/4
Professional:	1
Other:	1-1/4

Project Description:

Objectives:

1. To investigate the bacteriology of a transmissible periodontal pathosis which occurs in hamsters and to establish the identity of an etiologic agent.

2. To study the nutritional and metabolic activities of caries-active streptococci from the rat and hamster in order to discover some basis for their pathogenicity. This is a continuation of Project NIDR-13 (1961).

Methods Employed:

The methods employed are essentially those described in previous reports (NIDR-13, 1961). Standard bacteriological and biochemical techniques were employed for in vitro investigations on the metabolic activities of cariogenic streptococci. Observations on subgingival plaque and periodontal pathosis were made on live hamsters and were recorded photographically. Oral inoculations were made with broth cultures of the organisms under study. In some cases the germfree technique was employed.

Major Findings:

1. A periodontal syndrome in hamsters, characterized by heavy subgingival plaques, soft tissue recession and alveolar bone loss, could be induced in uninfected hamsters by oral inoculation with pure cultures of a filamentous organism. This organism was isolated from plaque in a naturally infected hamster. The disease was also produced by inoculation with a terramycin-resistant mutant strain of the filamentous organism. It could be recovered from the resulting plaque in high numbers on blood agar plates containing 1 μgm/ml terramycin. The condition produced by pure culture inoculation appeared to be identical to that produced in naturally infected animals. Histologic sections of periodontal structures showed the invasion of underlying soft tissue by filamentous elements of the plaque.

Germfree mice were monoinfected with the filamentous organism from hamster periodontal plaque. The organism became established in the intestinal tract and was consistently recovered in high numbers. Although gingival plaque was formed, there was no evidence of soft or hard tissue pathosis in the mice.

Lactate, acetate, formate and succinate were found to be the major end products of glucose metabolism by this organism.

2. Studies on the oleic acid sensitivity of cariogenic streptococci, reported last year, have continued. The saturated fatty acids stearic, palmitic, and elaidic reversed oleate inhibition of these organisms. Tween 80, lecithin, CO_2, sodium carbonate and sodium bicarbonate were also effective in this respect.

Sodium oleate inhibited glycolysis in resting cells of a caries-active hamster streptococcus.

Sodium oleate fed to hamsters at a dietary level of 0.5% had no effect on dental caries. Sodium stearate and hydrogenated coconut oil were also without effect.

Cariogenic streptococci from the rat and hamster were grown for the first time in a simplified, chemically defined medium. Under these growth conditions an absolute requirement for CO_2 was demonstrated.

3. Previously reported studies (NIDR-15, 1959) on the mode of action of sodium metabisulfite as an inhibitor of glycolysis were continued in collaboration with Dr. Narrod. This compound caused an increase in the production of CO_2, acetoin, and formic acid from glucose by a cariogenic rat streptococcus and a decrease in lactate. Preliminary studies showed the absence of the hexose

monophosphate shunt as an alternate metabolic pathway, indicating that the site of metabisulfite inhibition is in the classic Embden Meyerhoff scheme of glycolysis.

Significance to Dental Research:

Studies described in this report on a transmissible periodontal syndrome in hamsters have a very real significance in that they should provide a model system for the study of periodontal disease in general. With this experimental system it may be possible to study the significance of other factors thought to be of importance in periodontal disease. A more intensive study of the etiologic agent described may provide valuable information on the microbial factor in periodontal disease.

The in vitro bacteriological studies on pure strains of caries-producing streptococci are of both practical as well as theoretical value. Biochemical differences between these organisms and certain noncariogenic strains may be exploited for the selective detection and cultivation of these active strains. Blocking the particular enzymatic steps of significance in caries causation by the use of selective inhibitors may have therapeutic possibilities. More importantly, a knowledge of the differences between cariogenic streptococci and other noncariogenic types may provide important clues for understanding the basic reasons for their dental pathogenicity.

Proposed Course of Project:

Studies on the induction of periodontal pathosis by pure cultures of the filamentous organism will continue. Using the technique of inoculation of uninfected hamsters with pure cultures of the terramycin resistant "labeled" strain it will now be possible to study the influence of other factors like diet and the age and physical condition of the host on this condition. Of immediate concern to us are the factors which govern transmissibility and implantation of the etiologic agent in the host animal. Also, the importance of other components of the microbial flora will be investigated.

Development of a chemically defined medium for the growth of caries-active streptococci now makes it possible to study nutritional requirements in more detail. Amino acid, vitamin, purine and pyrimidine requirements will be determined. The metabolic significance of the essential CO_2 is under investigation.

Continuing studies on the oleate sensitivity of the cariogenic streptococci will be directed toward discovering the mode of action of this bacterial inhibitor.

Part B included Yes

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Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Jordan, H. V., Fitzgerald, R. J., and Berger, N. D. A Comparison of the Caries Inhibitory Potential of Sodium Metabisulfite and Related Compounds. J. Dental Res. 41: 61-65, 1962.

Keyes, P. H., Fitzgerald, R. J., Jordan, H. V., and White, C. L. The Effects of Various Drugs on Caries and Periodontal Disease in Albino Hamsters. Arch. Oral Biol. Special Suppl. 7: 123-141, 1962.

Honors and awards relating to this project:

None

Serial No. NIDR-7
1. Microbiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1962

Part A

Project Title: The Biochemistry of the Differentiating Cellular Slime Mold, Dictyostelium discoideum.

Principal Investigator: Dr. M. I. Krichevsky

Other Investigators: None

Cooperating Units: Department of Chemistry, Howard University, Washington, D. C,, Dr. William Hills

Man Years (calendar year 1962):

Total:	2-1/2
Professional:	1
Other:	1-1/2

Project Description:

Objectives:

This is a continuation of Project NIDR-12 (1961), to investigate the biochemical factors initiating and controlling morphogenesis of Dictyostelium discoideum. Presently the main effort is concerned with the factors affecting the rate of differentiation but not the quality. Also a study is in progress concerning the nature of the pigments of D. discoideum and D. purpureum. The pigments are apparently contained only in the mature spores. The stalk cells remain colorless. Thus we have a qualitative rather than quantitative difference between the two cell types facilitating study of factors controlling the appearance of cellular differences.

Methods Employed:

All are standard techniques routine to the types of studies herein described.

Major Findings:

The amino acid, histidine, has been found to stimulate the rate of morphogenesis at pH5 but not at pH7. Little or no difference

was found in the amount of histidine taken up by the amoebae at these pH values. It is concluded that the histidine outside or at the surface of the cells is the controlling factor rather than the internal histidine.

It was found that histidine decreases the ability of exogenously supplied amino acids to penetrate the internal free pool. In addition it was found that histidine decreased the amount of ultraviolet-absorbing material leaving the cells. A direct correlation was found between the ability of materials to stimulate morphogenesis and their ability to prevent the leakage from the cells of ultraviolet-absorbing material.

A metabolite of histidine by this organism has been tentatively identified as urocanic acid. However, this metabolite is known to be unable to stimulate morphogenesis.

The leakage of protein has been studied and found not to be effective in the same manner (e.g., histidine does not prevent leakage of protein into the medium). Therefore it is concluded that the leakage is a function of the whole population of cells rather than due to the nonspecific damage of a small number of cells.

The yellow pigments of D. discoideum have been isolated by simple ethanol extraction. Two pigment fractions have been found. One is a polar fraction soluble in ethanol but not chloroform. This fraction has three components as indicated by thin-layer chromatography on silica gel. The second fraction is nonpolar since it is soluble in chloroform as well as ethanol. This group of pigments exhibits eight components when subjected to thin-layer chromatography.

All eleven substances are thought to be closely related, since the ultraviolet and infrared absorption spectra of chromatographically purified materials all have similar characteristics. The infrared spectra indicated the presence of carbonyl functions (such as carboxylic acids, esters, amides, etc.). The differences among the compounds appear to be due to differing numbers of such groups in the various compounds.

The purple pigment of D. purpuereum has resisted isolation to date. The pigment appears to be chemically bound to the spores since simple solvent extraction by polar or nonpolar solvents does not give a discernible amount of dissolved pigment. Treatment with alkali yields a slime that is difficult to handle. Mild acid hydrolysis leads to profound changes in the pigment. Experiments are in progress to determine the feasibility of enzymatic hydrolysis prior to extraction. Various cellulases and other hydrolytic enzymes are being tested.

Significance to Dental Research:

The phenomena of nongenetic functional changes in cell types, such as embryological differentiation, microbial spore formation, adaptive enzyme formation, cancerous de-differentiation, aging, etc., are an integral and fundamental part of the life history of all organisms. It is desirable to have information regarding the biochemical factors influencing the initiation and course of such changes since many aspects of medical phenomena are intimately affected by these functional differences among cells.

The cellular slime molds have several advantages as a model system for such studies:

1. The amoebae, which are a uniform population in the vegatative stage, form two and only two differentiated cell types (i.e., spore cells and stalk cells).

2. They are simple to grow in quantity under routine laboratory conditions.

3. The formation of the two cell types takes place under starvation conditions, thereby allowing close nutritional control of the process. In addition, the cellular differentiation is unconfounded by associated phenomena due to growth.

4. The spore mass is attached to the stalk only by surface tension, thus the separation of the two is facilitated.

Proposed Course of Project:

Future experiments will be designed to test the effect of a variety of compounds on the intracellular adenosine triphosphate levels of differentiating cells to ascertain whether the mechanism of action whereby these materials prevent leakage of cellular components into the medium is the production or conservation of adenosine triphosphate.

A study of the nutritional requirements of the vegatative amoebae will be undertaken. A defined medium is required so that the initiation of differentiation can be closely controlled and studied.

The study of pigmentation of the slime mold will involve determination of: a) more refined purification procedures, b) chemical structures, c) nutritional factors in biosynthesis, d) pathways of biosynthesis, and e) factors controlling the formation of biosynthetic enzymes.

Part B included No

Serial No. NIDR-8
1. Microbiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1962

Part A

Project Title: Role of Genetic, Microbiological, Nutritional and Host Factors on Experimental Dental Caries

Principal Investigator: Dr. R. H. Larson

Other Investigators: Dr. R. J. Fitzgerald

Cooperating Units: None

Man Years (calendar year 1962):

Total:	2-1/4
Professional:	1
Other:	1-1/4

Project Description:

Objectives:

This is a continuation of Project NIDR-10 (1961), to evaluate the separate roles of host animal, diet and oral flora in the development of caries in different strains of rats.

Methods Employed:

Conventional methods for animal experimentation have been utilized. Caries activity has been compared in two strains of animals, and their hybrid offspring. The studies have involved the breeding of more than 1,000 animals in our laboratory, more than 800 of which have been subjected to caries test periods of 2 to 3 months. A complete pedigree has been maintained for each animal bred, together with all changes in diet and a record of antibiotic or other special treatment.

Major Findings:

1. The NIH Black rat has been shown to be less caries active than the Osborne-Mendel rat, thus providing two strains of animals in which various host factors could be studied. The first phase of the study has been to compare the caries activity of the two strains with the various hybrid offspring, giving special consideration to

any differences which may have resulted from transmitted flora.

The Black rat has consistently shown a greater resistance to caries under all the experimental conditions imposed than has the Osborne-Mendel rat. It has not been possible to enhance caries activity in the Black rat by caging during the caries-test period with an Osborne-Mendel rat nor by subjecting the mother to a caries-test period before breeding. Both methods have been effective with Osborne-Mendel rats. A slight increase in activity was shown in Black rats pretreated with an antibiotic and caged with an older caries-active animal at the beginning of the caries-test period.

The hybrid animals developed more caries activity and were more susceptible to increased caries-activity by transmission than the Black rat. The offspring of Osborne-Mendel females bred to a Black male developed a level of activity between that of the two parent strains. Their activity was not enhanced when they were caged with Osborne-Mendel animals during the caries-test period. On the other hand, hybrid animals from Black mothers and Osborne-Mendel fathers were no more active than the Black rats when run separately. However, those caged with Osborne-Mendel rats during the caries-test period developed caries comparable to hybrids of Osborne-Mendel mothers.

2. There are reports in the literature that rats born of mothers on a high sugar diet are more caries susceptible than those of mothers on a low sugar diet. No consideration was given to possible effects of such diets on the oral flora of the mother and its transmission to her offspring. In a pilot study one group of animals were bred on a semi-purified caries-test diet and another group on a similar diet which contained corn starch instead of confectioner's sugar. When animals from the two dietary groups were caged together during the caries-test period their activities were comparable. These results suggest that the offspring of mothers on the sugar diet were no more susceptible than those on the starch diet, but may have acquired a more highly active cariogenic flora from their mothers.

Significance to Dental Research:

Comparative studies of the NIH Black rat with the Osborne-Mendel rat and their hybrid offspring demonstrated the importance of both genetic background and transmissible flora in experimental caries. The caries activity of the control hybrid animals was closely related to that of their mothers. Animals which were exposed to caries-active animals developed activity directly related to the genetic background. Contrary to previous reports in the literature, the study of prenatal and lactating diets of the mother suggests that there is no difference in tooth susceptibility of the offspring

related to starch vs. sugar as the major component in the diet. This study points out the importance of attempting to equalize the flora of animals with different experimental backgrounds before suggesting that a given regimen has produced a difference in caries susceptibility per se.

Proposed Course of Project:

It has been shown in this laboratory that there is a dramatic decrease in caries activity with increased age of the rat, and that this decrease is greatest on the bucco-lingual surfaces of the teeth. Plans are underway for a cooperative study to investigate the role of several factors which may be involved in the aging process, for example, changes in gingival tissue, salivary secretions, oral ecology, chemical composition and crystalline structure. Variations in caries activity have been demonstrated with different strains of animals and with animals of different ages, etc. It has been impossible by present methods to determine the extent to which these differences have been due to actual differences in tooth susceptibility. An *in vitro* system for testing caries susceptibility of the teeth would be highly advantageous. Work has been started on a method which it is hoped will give some answer in this area.

Genetic differences have been demonstrated between the Osborne-Mendel and NIH Black rats. It has not been shown to what extent these differences may have been dependent on the ability of the animals to support the available "cariogenic" flora while being fed a specific caries-test diet. It is our plan to expose the Black rat to other strains of caries-active animals and also to determine their response to different caries-test diets.

In addition, studies of the electrophoretic patterns of the saliva of the different strains are being made.

Part B included Yes

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Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Larson, R. H., Rubin, M., and Zipkin, I. Frequency of eating as a factor in experimental dental caries. Archives of Oral Biology 7: 463-468, 1962.

Larson, R. H., Zipkin, I., and Fitzgerald, R. J. Effect of dehydroacetic acid and Tetrocycline on caries activity and its transmission in the rat. Accepted for publication in J. Dent. Res.

Larson, R. H. A semi-purified caries-test diet for rats. Accepted for publication in J. Dent. Res.

Honors and awards relating to this project:

None

Serial No. NIDR-9
1. Microbiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1962

Part A

Project Title: Mechanisms of Action of Oral Bacteria and Their Products in Oral and Systemic Disease.
1. Biological, Physical, and Immunochemical Studies on Endotoxins and Other Antigens from Oral Bacteria.
2. Mechanisms of Host Resistance and/or Susceptibility to Bacteria Indigenous to the Human Oral Cavity.
3. Effect of Oral and Intestinal Flora on Susceptibility of Mice to Bacterial Endotoxin.

Principal Investigator: Dr. S. E. Mergenhagen

Other Investigators: Dr. S. B. Jensen
Dr. W. Chagas de Araujo
Dr. H. V. Jordan

Cooperating Units: Laboratory of Biochemistry, NIDR
Laboratory of Biochemical Pharmacology, NIAMD

Man Years (calendar year 1962):

Total:	4-1/2
Professional:	2-1/4
Other:	2-1/4

Project Description:

Objectives:

This is a continuation of Project NIDR-11 (1961), with the following immediate objectives:

1. To determine which members of the oral microbiota possess endotoxins or other antigens capable of inciting tissue damage directly or by hypersensitivity mechanisms.

2. To analyze, by physical and immunochemical techniques, the nature of such antigens extractable from oral bacteria.

3. To analyze the mechanism of action of endotoxins and other bacterial products on host tissue, i.e., to investigate the role of endotoxins from gram-negative bacteria in increasing resistance

or susceptibility to experimental infection with oral or other bacteria, and to study the effect of the indigenous oral and intestinal flora on susceptibility of the host to bacterial products.

Methods Employed:

The two-phase phenol-water extraction of Westphal has been applied to oral and other bacteria, to release soluble endotoxins from the cells. Toxicity of isolated bacterial fractions was tested by intracutaneous and intravenous injection in rabbits and intraperitoneal injection in mice. Pyrogenic activities of endotoxin preparations were tested in rabbits. Development of lesions from primary injections in rabbits was followed grossly and histologically. Similar observations were made on rabbits given a second injection intravenously to elicit the localized Shwartzman reaction. Toxicity for mice was calculated as the 50 percent lethal dose. In addition, the effects of endotoxin on oral tissues were investigated by injecting these preparations intramucosally in the gingival-palatal area of the rabbit. Both direct toxicity to oral mucosa and Shwartzman reactions in oral mucosa were followed histologically. The effects of intramucosal injection were further studied by measuring systemic temperatures and examining regional lymph nodes draining the injected oral sites. Chemical and physical analysis of these lipopolysaccharide endotoxins utilizes paper chromatography and specific colorimetric tests for polysaccharide elements, ultracentrifugation for purification of endotoxins and to determine the molecular properties of the material, and chemical fractionation of the endotoxin to determine whether the toxic moiety is really only a part of the whole complex isolated by phenol-water method.

Immunological analyses of lipopolysaccharides and protein antigens are being carried out by using immunoelectrophoresis, double diffusion techniques, agglutination, hemagglutination, and hemagglutination-inhibition techniques. Biological analysis for specificity of veillonella endotoxins has been performed by testing the effect of prior experience with living or dead bacteria on the response of mice and rabbits to endotoxins.

An evaluation is being made on the effect of the oral and intestinal microbial flora in sensitizing the host to bacterial lipopolysaccharides. This study entailed the use of germfree mice and specific pathogen-free (SPF) mice kept in isolators in the Gnotobiotic Section.

Major Findings:

1. (a) Endotoxins have been extracted by the phenol-water procedure from oral strains of Veillonella, Selenomonas sputigena,

Bacteroides melaninogenicus, Fusobacterium nucleatum, Leptotrichia, small oral treponemes, Borrelia vincentii and Borrelia bucallis, but were not found in viridans streptococci. These endotoxins produced intracutaneous inflammatory necrotic lesions, prepared skin sites for the local Shwartzman reaction, were pyrogenic in rabbits, and were lethal for mice. In addition, the endotoxin from Veillonella strains (others not tested) produced inflammatory necrotic lesions in the oral mucosa of the rabbit and prepared this tissue for the local Shwartzman reaction. These effects were obtained with as little as 1 microgram of endotoxin. A comparison of the response of oral mucosa and of skin to similar local injections of endotoxin revealed that the gross and histologic manifestations following this specific type of insult were basically similar in both tissues. It was observed, however, that an intramucosal injection of 1 microgram produced temperature elevation, whereas 25 times this dose given intracutaneously was not pyrogenic.

1. (b) The lipopolysaccharide-containing endotoxin fraction, isolated by the phenol-water procedure from veillonella cells, has been characterized chemically as follows: it contained 10 percent Kjeldahl nitrogen, 4 percent protein, 4.2 percent phosphorus, 60 percent chloroform-soluble lipid after acid hydrolysis, 48 percent nucleic acid.

Nucleic acid contamination of the above endotoxins could be eliminated by preliminary treatment of the bacterial cells with 0.5 percent formol or by ultracentrifugation of the dialyzed water phase after phenol-water extraction. This procedure proved useful for getting highly toxic preparations free of nucleic acid. Veillonella endotoxin so prepared had low contents of nitrogen (0.4 percent), phosphorus (0.5 percent) and protein (1.8 percent). Concomitant with the reduction in these chemical components, the minimum lethal dose (LD_{50}) for mice and the minimum effective preparatory dose for the local Shwartzman reaction were reduced. Analytical ultracentrifuge data indicate that this endotoxin is polydisperse and has several components of varying molecular weight. However, products separated in a Sephadex column indicate that the fractions with the greatest molecular weight (>50,000) are responsible for the toxicity of the whole fraction tested. Polysaccharide components thus far identified in veillonella endotoxin have been glucosamine, galactosamine, sialic-acid like components, and a fast moving hexose with chromatographic properties similar to 3-methylglucose. The hexosamine components were identified colorimetrically and by ion-exchange chromatography. Sialic acid was identified by the thiobarbituric acid assay method and also by paper chromatography with known references of N-acetylneuraminic acid and N-glycolyneuraminic acid. Sialic acid-like materials have been found also in the endotoxin of Selenomonas sputigena but not in the others.

1. (c) Utilizing hemagglutination techniques and double diffusion techniques, an immunochemical study of Veillonella, Fusobacterium, and Leptotrichia antigens demonstrated that the endotoxic moieties are in part responsible for the specific agglutinating properties of the above-named organisms, i.e., they are part of the mosaic of surface antigens. Veillonella lipopolysaccharide proved to be a poor antibody-producer in vivo; however, it could be used as a hapten in hemagglutination tests, in which rabbit antiserum to whole cells agglutinated endotoxin-sensitized sheep erythrocytes. Biologically active (pyrogenic, Shwartzman-reactive) lipopolysaccharides were prepared by phenol-water extraction of nineteen strains of oral anaerobic gram negative cocci conforming to the characteristics of the genus Veillonella. Analyses of certain of these lipopolysaccharides for lipid, protein, polysaccharide, nitrogen and phosphorus contents revealed a striking similarity in gross chemical composition. Conventional agglutination tests and hemagglutination tests employing lipopolysaccharide-sensitized sheep erythrocytes compared favorably as a means of determining the potency of anti-veillonella rabbit sera. The lipopolysaccharides appeared to be serologically specific as determined by hemagglutination-inhibition tests, which established several serotypes of veillonella. This specificity could not be demonstrated by conventional agglutination tests or by agargel diffusion tests using cell extracts.

Similar observations on the specificity of lipopolysaccharides are being made on purified endotoxins from strains of Fusobacterium and Leptotrichia. In addition studies on the antigens from protoplasmic fractions and cell wall fractions of Fusobacterium polymorphum indicate a possible separation of "endotoxic material" into polysaccharide haptens and toxic lipopolysaccharide. Such antigens are being assessed in double diffusion tests.

2.(a) Vaccination with viable or heat-killed oral anaerobic gram-negative cocci (genus Veillonella) increased the resistance of NIH Swiss Webster mice to lethal effects of veillonella endotoxin. Protection was best conferred by vaccination with the strain of Veillonella from which the serologically specific endotoxin was prepared. Utilizing weight loss following sublethal dosages as a measure of biological effects of endotoxin, the duration and extent of weight loss in vaccinated mice is also considerably reduced as compared to nonvaccinated animals. Vaccination with heat-killed veillonellae significantly protected Bordetella pertussis-sensitized mice against veillonella endotoxin, but not against lethal doses of Escherichia coli endotoxin, histamine, or serotonin. Vaccination of mice with another oral gram-negative bacterium (Fusobacterium) did not protect pertussis-sensitized mice against the lethal effect of veillonella endotoxin.

2.(b) Intraperitoneal injection in mice of mixed cultures of oral

bacteria as contained in suspensions of material from the human gingival sulcus leads to a severe bacteremia with viridans streptococci. Eighty to 100 percent of such animals succumb within 24 hours. Streptococci alone in suspensions of the same optical density as the mixed cultures are not lethal to mice, but injection of sublethal amounts of heat-killed fusobacteria or veillonellae immediately prior to challenge with streptococci results in a ten-fold decrease in LD_{50}. Pretreatment with heat-killed gram-negative oral bacteria or sublethal microgram quantities of their purified lipopolysaccharides increases susceptibility to oral streptococci during the first 12 hours, followed by a period of increased resistance after 24 hours. This biphasic effect of endotoxin on the resistance of mice to peritoneal infection with streptococci has also been substantiated by observations on the duration of the ensuing bacteremia. The stimulating effect of endotoxin on nonspecific resistance observed after 24 hours has been shown to depend not only on an increased blood clearance by the reticulo-endothelial system but also on increased phagocytic properties of mononuclear and polymorphonuclear cells residing in the peritoneal cavity of endotoxin-treated mice. In vitro experiments are continuing to study interactions between oral bacteria and the inflammatory cells (polymorphonuclear leucocytes and mononuclear leucocytes) that may be found in the gingival sulcus.

2.(c) In addition to the above outlined studies on oral bacteria, the lipopolysaccharide endotoxin of group C Neisseria meningitidis, strain 1908, has been isolated by phenol-water extraction and further purified by ultracentrifugation of the water soluble lipopolysaccharide-nucleic acid complex. The lipopolysaccharide was found to contain 20 percent lipid (composed of cephalin, fatty acids, and plasmalogen) with polysaccharide constituents identified as glucose, galactose, glucosamine, and sialic acid. The endotoxin contained less than 1 percent protein. Hemagglutination inhibition tests revealed that meningococcal lipopolysaccharide was able to inhibit antibodies from agglutinating sheep erythrocytes sensitized with type-specific capsular polysaccharide, which is composed mainly of units of sialic acid. The converse was not found however. Analytical ultracentrifugation and the separation of lipopoly-saccharide and of capsular polysaccharide by gel filtration (Sephadex) provided evidence that the sialic acid formed an integral part of the biologically active lipopolysaccharide. "Crude" and "purified" lipids extracted from the parent lipopolysaccharide failed to demonstrate lethal activity in high dosages in normal or pertussis-sensitized mice. They were found to be nonpyrogenic and nonpreparative for the local Shwartzman reaction in rabbits in comparison to the native lipopolysaccharide. In rather small dosages, however, they were capable of increasing nonspecific resistance in the mouse to a lethal Escherichia coli infection.

The role of lipid from meningococcal endotoxin in stimulating

resistance in the mouse to such organisms as Escherichia coli, Salmonella typhosa, and Staphylococcus aureus prompted a further study on the mechanism whereby lipid from lipopolysaccharide induces host resistance. Similar to the findings of Westphal with "Lipid A" component, we observed no negative resistance phase in mice after treatment with meningococcal lipid. Interestingly enough, we have demonstrated that this lipid is nontoxic and is also effective in rabbits as well as mice in protecting the host against a fatal coliform infection. Livers and spleens from lipid-treated mice have increased ability to clear E. coli after intravenous injection. This finding points to a prominent role of the reticuloendothelial system in this induced resistance. In contrast to the parent lipopolysaccharide, however, lipid is incapable of inducing immunity to a challenge with graded doses of E. coli endotoxin. In vitro experiments have shown that mouse liver homogenate is capable of liberating biologically active lipid from meningococcal lipopolysaccharide. That is, chloroform-soluble lipid, released by the action of mouse liver homogenate on lipopolysaccharide, stimulates host resistance after injection in the mouse. The use of isotopically (C^{14})-labelled meningococcal lipopolysaccharide, prepared from cells grown in trypticase soy medium with C^{14}-labelled acetate, provided additional evidence of the hydrolysis of lipopolysaccharide by mouse liver. Incubation of isotopically-labelled lipopolysaccharide with mouse liver homogenate released chloroform-soluble radioactive lipid. At this point we are postulating a possible mechanism whereby lipopolysaccharide induce host resistance. Once the lipopolysaccharide is taken up by liver tissue and possibly others, hydrolysis by enzymes present in the tissue releases lipid which stimulates phagocytosis by the reticuloendothelial system. The remaining polysaccharide moiety may be responsible for two events in the host: firstly, a stimulation of enzymes capable of detoxification and, secondly, the induction of specific bactericidal antibodies which may be operative against gram-negative bacteria.

3. Repeated experiments have shown that germfree mice are significantly more resistant than conventional mice to challenge with endotoxins isolated from coliform bacteria residing in the intestine of their conventional counterpart. Whereas no difference was found in the reaction of germfree Lobund and germfree NIH mice to endotoxin, the conventional NIH mice were generally more susceptible than the conventional Lobund mice. A survey of the intestinal flora showed that the NIH mouse harbored 10 times as many coliform organisms in its intestinal contents as the Lobund mouse. Streptococci and lactobacilli were also much more numerous in feces samples from NIH mice than from Lobund mice. Initially germfree mice subjected to the external environment for three weeks acquired an intestinal flora comparable to that of the conventional mouse and such animals were equally as susceptible to challenge with endotoxin. Monoinfection of germfree mice kept

in isolators did not affect their susceptibility to endotoxins as compared to germfree mice. Whereas SPF (specific pathogen free) mice, when kept in isolators, are quite resistant to coliform endotoxin, the superimposing of coliform bacteria on their existing intestinal flora (streptococci, lactobacilli, pseudomonads) makes them just as susceptible to endotoxin challenge as their conventional counterparts, or even more so.

Significance to Dental Research:

The demonstration by chemical and biological means of endotoxins in certain strains of oral bacteria, especially those which are found in greater abundance in the oral cavity in pathologic processes of oral soft tissues, supports the frequently advanced speculation implicating toxic components of the oral microbiota in the inflammatory, necrotic phases of periodontal disease. The demonstration of a mechanism whereby minute quantities of lipopolysaccharide, possibly derived from bacteria residing in the gingival sulcus, effect host resistance or susceptibility to infection may implicate components of the oral flora in systemic disease. The finding of specific antigens in gram-negative oral bacteria by immunological and biological techniques may contribute to a more complete understanding of the host-parasite relationship in oral and systemic disease. Evidence for the susceptibility of gingival tissue to endotoxins and to other antigens in hypersensitized animals may implicate the allergenic properties of oral bacteria in oral disease.

Proposed Course of Project:

To continue our investigations as outlined above with a particular reference to mechanisms of action of endotoxin and other bacterial products on host tissue, accessibility of oral tissues to such bacterial products, and a physical, biological, and immunochemical study of endotoxins and other antigens from gram-negative bacteria.

Part B included Yes

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Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Mergenhagen, S. E., Zipkin, I. and Varah, E. Immunological and Chemical Studies on an Oral Veillonella Endotoxin. J. Immunology, 88: 482-487, 1962.

Mergenhagen, S. E., and Jensen, S. B. Specific Induced Resistance of Mice to Endotoxin. Proc. Soc. Exp. Biol. and Med. 110: 139-142, 1962.

Mergenhagen, S. E. and Varah, E. Serologically Specific Lipopolysaccharides from Oral Veillonellae, Arch. Oral Biology, In press, 1962.

Mergenhagen, S. E., Martin, G. R., and Schiffman, E. Studies on an Endotoxin of a Group C Neisseria meningitidis. J. Immunology, In press, 1962.

Honors and Awards Relating to this Project:

None

Serial No. NIDR-10
1. Microbiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1962

Part A

Project Title: The Relationship Between Tissue Enzymes and Experimental Infection

Principal Investigator: Dr. A. L. Notkins

Other Investigators: None

Cooperating Units: National Cancer Institute, Dr. Robert E. Greenfield

Man Years (calendar year 1962):

Total:	2-1/4
Professional:	1
Other:	1-1/4

Project Description

Objectives:

This is a continuation of Project NIDR-3 (1961). Although a vast amount of work has been done on the relationship between infection and antibody titer, there are relatively few studies on the enzyme changes which take place in the infected animal. Such studies are of utmost importance in understanding cell damage and the biochemical and genetic alterations which occur in the infected cell. Because of its pertinence to these problems the lactic dehydrogenase elevating agent (LDH agent) has been studied extensively. We have been concerned with (1) the qualitative and quantitative changes in enzyme activity produced by this agent in the normal and experimentally altered animal, (2) the growth characteristics and properties of this agent, and (3) its mode of action.

Methods Employed:

Conventional microbiological and pathological techniques are employed for the transmission and study of infection in mice. Plasma is prepared from blood collected by orbital bleeding, and analyzed for enzymatic activities by established methods. Assay for the LDH agent depends upon demonstration of a significant rise in the plasma LDH activity.

Major Findings:

While studying the relationship between tumor growth and plasma enzyme activity, Riley et al.(Science, 132: 545, 1960) found a transmissible agent in the plasma of many tumor-bearing mice which, upon injection into normal mice, produced a 5- to 10-fold increase in the plasma activity of the enzyme lactic dehydrogenase (LDH). The increase in plasma LDH occurred within 72 hours after injection and the elevation persisted for months despite the lack of any gross pathology. Initially, Riley studied 26 different types of mouse tumors and recovered a transmissible agent from all of these tumors. Because of this high recovery, the relationship of the LDH agent to the tumor process was of considerable interest. Experiments conducted in our laboratory showed that not all tumors contained this agent. In addition we showed that the Moloney leukemia agent could be separated from the LDH agent by passage through rats without the Moloney agent losing its ability to produce leukemia. This and other evidence suggested that the LDH agent was not integrally related to the tumor process but was a contaminant which was being carried by many serially transplanted mouse tumors.

In addition to the enzyme LDH our studies showed that isocitric dehydrogenase (ICDH), malic dehydrogenase (MDH), phosphohexose isomerase and glutamic-oxalacetic transaminase were also elevated within 72 hours after injection of the LDH agent and remained elevated for months. Because of the wide contamination of experimental tumors and the multiple enzyme-elevating capacities of this agent, it was decided to reinvestigate the relationship between tumor growth and the increase in plasma enzyme activity by employing a mouse tumor which was free of the LDH agent. These studies revealed that (1) the early rise in plasma enzyme activity prior to the actual appearance of the tumor was not due to the tumor but was the result of infection with the LDH agent and (2) plasma LDH, MDH, and ICDH of the infected tumor-bearing animal was many times higher than that of the uninfected tumor-bearing animal. In addition, the plasma level of the enzyme LDH was several times greater in the infected tumor-bearing animal than could be accounted for by simply adding the plasma enzyme activity of the normal animal infected with the LDH agent to that of the uninfected tumor-bearing animal. A similar, although less pronounced, increase was also seen with MDH. Lastly, uninfected tumor-bearing mice showed a late increase in plasma enzyme activity which appeared to be related to tumor growth. From these studies it became clear that much of the increased plasma enzyme activity previously attributed to tumor growth was in fact the result of contamination of the tumor with the LDH agent.

The above findings pointed to the necessity of characterizing the LDH agent. An assay method for determining the infective titer of

the LDH agent was worked out and the growth curve of this agent was studied. Within 24 hours after infection, a titer of $10^{10.8}$ I.D.50/ml. was found in the plasma of mice. A viremia existed for as long as one year after infection with a titer of $10^{4.3}$ I.D.50/ml. at the end of 12 months. Experiments concerned with the physical and chemical properties of the LDH agent showed that it was inactivated by ether but withstood lyophilization and freezing and thawing. It was stable over a wide pH and temperature range. Ultracentrifugation at 105,000 x G for 2 hours left less than 1% of the LDH agent in the supernatant fluid and Gradocol membrane filtration studies suggest that the upper size limit of the LDH agent is 72 mμ. Electron microscopic studies are in progress with Dr. Howard Bladen of this Institute. The spread of the LDH agent from infected mice to uninfected mice kept in the same cage was relatively low and recent experiments have shown that the LDH agent can be recovered from the saliva, feces, and urine only early in the course of infection. At the present, attempts are being made to isolate an infectious nucleic acid.

From our studies and those in the literature, there appears to be little doubt that the LDH agent is a virus. It is characterized by a number of unusual properties including (1) rapid multiplication associated with a high plasma titer, (2) a viremia lasting for at least one year (suggesting a poor antibody response), (3) prolonged elevation of a number of plasma enzymes, and (4) the lack of gross pathology or illness.

Significance to Dental Research:

The studies undertaken in this laboratory have been concerned with a) the qualitative and quantitative changes produced by the LDH agent in the normal and tumor-bearing animal, b) the characteristics of this agent and c) the mechanism by which the LDH agent produces these changes. Through studies of this type we hope to get a closer understanding of the basic mechanism involved in infection, the development of new parameters by which to study these phenomena, and ultimately to apply our findings to the clinical area of oral infection and neoplasia.

Proposed Course of Project:

Characterization of the LDH agent will be continued and the mechanism of enzyme elevation will be studied in more detail. Other parameters of experimental infection and enzyme alteration will be investigated.

Part B included Yes

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Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Notkins, A. L., Berry, R. J., Moloney, J. B., and Greenfield, R. E. Relationship of the Lactic Dehydrogenase Factor to Certain Murine Tumors. Nature 193: 79-80, 1962.

Notkins, A. L., and Greenfield, R. E. Infection of Tumor-bearing Mice with the Lactic Dehydrogenase Agent. Proc. Soc. Exptl. Biol. Med. 109: 988-991, 1962.

Notkins, A. L., Greenfield, R. E., Marshall, D., and Bane, L. Multiple Enzyme Changes in the Plasma of Normal and Tumor-bearing Mice Following Infection with the Lactic Dehydrogenase Agent. J. Exptl. Med., in press.

Honors and Awards relating to the project:

None

Serial No. NIDR-11
1. Microbiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1962

Part A

Project Title: The Role of Bacteria in the Formation of Dental Calculus

Principal Investigator: Dr. A. A. Rizzo

Other Investigators: Dr. J. M. Lifschiz
Dr. D. B. Scott
Dr. H. Bladen

Cooperating Units: None

Man Years (calendar year 1962):

Total:	1
Professional:	1/2
Other:	1/2

Project Description:

Objectives:

1. To discover the role of bacteria in the development of mineralized deposits on teeth.

2. To investigate, specifically, the capacity of individual oral organisms to serve as a matrix for calcification and the capacity of individual oral organisms to bring about adherence of the calcified deposits onto teeth.

Methods Employed:

Washed pure cultures of organisms isolated from calcifying human dental plaques were transferred into sealed dialysis bags and implanted in the peritoneal cavities of rats. Nonviable organisms and several other substances were implanted in the same manner. In studies on adherence, viable organisms and segments of lower incisor teeth from young adult rabbits were implanted together. These tooth specimens were used because they were of convenient size, of uniform shape, and of uniform surface texture. In order to make sure that no organic deposits were present before implantation, the teeth were either boiled or papain treated, were washed and were then examined under magnification. Animals were sacrificed after varying

intervals up to 1 and the contents of the bags were subjected to gross examination, bacteriological procedures, x-ray diffraction, electron microscopy, and electron diffraction.

Major Findings:

As indicated in a previous annual report (Project NIDR-9, 1961), ten different species of oral organism have been shown to bring about calcification in 13-26 days. Electron microscope studies show that the bacterial cell body itself serves as a focus for beginning crystallization. In some species the calcification seems to begin within the cytoplasm of the organism in a true intracellular manner, whereas in other organisms, mineralization begins at the surface of bacteria.

Studies on the attachment of the calcified deposits onto teeth are not yet completed. However, in preliminary experiments, grossly visible hard deposits resembling clinical calculus became attached to teeth implanted with Actinomyces israelii for several months, whereas no attachment of deposits occurred on teeth implanted with an oral diphtheroid for 6 months.

An interesting incidental finding has been that viable organisms in an implanted dialysis bag survive for long periods. A number of organisms have been recovered after more than 6 months inside the peritoneal cavity and recently the original organism from a 14-month old specimen was grown out in pure culture and identified.

Significance to Dental Research:

These studies have shown that oral organisms of several types may serve as a calcifiable matrix and that calcification occurs in intimate association with the bacterium itself. Although many workers have emphasized the role of salivary constituents in the formation of dental calculus, these findings strongly suggest that the role of bacteria in the development and retention of calcified deposits is highly important.

Proposed Course of Project:

1. It is planned to continue experiments in which bacteria and teeth have been implanted together in order to observe whether or not deposits become adherent.

2. Additional organisms, bacterial products, salivary constituents and other substances will be tested for calcifiability and for their influence on adherence.

3. In order to assay better the clinical significance of the findings, attempts will be made to reproduce representative findings in the human mouth.

Part B included Yes

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Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Rizzo, A. A., Martin, G. R., Scott, D. B., and Mergenhage, S. E. Mineralization of Bacteria. Science 135: 439-441, 1962.

Honors and Awards relating to this project:

None

Serial No. NIDR-12
1. Microbiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1962

Part A

Project Title: Experimental Ulcer Formation in Oral Mucosa

Principal Investigator: Dr. A. A. Rizzo

Other Investigators: W. K. Ashe

Cooperating Units: None

Man Years (calendar year 1962):

Total:	1-1/2
Professional:	3/4
Other:	3/4

Project Description:

Objectives:

1. To study intraoral ulcer formation and prevention in an experimental animal system.

2. To test viral, bacterial, dietary, and other substances, including human aphthous ulcer material, for their ulcerogenic capacity in the oral mucosa of the rabbit.

Methods Employed:

Herpes simplex virus (originally isolated from a herpes labialis vesicle of a white male adult) was inoculated superficially into a palatal site in the oral mucosa of rabbits. The development and fate of the lesion produced were studied by gross inspection, by microscopic examination, and by culture throughout the clinical course of the lesions. Using the pattern of cultivability of these experimental herpetic lesions as a basis, it was possible to select the optimum clinical stage of human aphthous ulcers most likely to yield positive viral cultures. Therefore, attempts were made to culture herpes simplex virus from a small number of developing human aphthous ulcers.

Intramucosal inoculations were also made of the following bacteria: Streptococcus salivarius, S. faecalis, S. mitis, Veillonella sp.,

Neisseria sp., a diphtheroid, Fusobacterium polymorphum, Actinomyces israellii, A. naeslundii, Bacterionema matruchotii, and Staphylococcus aureus. Tests were also made on the ulcerogenic capacity of human aphthous ulcer material and an orange peel extract (a possible chemical provocant of human aphthous ulcers).

In studies of prophylaxis against herpetic mucosal ulcers, animals were challenged intraorally at intervals after repeated parenteral inoculation, and also after a single intramucosal administration of herpes simplex virus. Blood was taken before these challenges, and herpes antibody assayed by neutralization techniques. In another experiment on prophylaxis, 5-iodo-2'-deoxyuridine (IDU which was reported to cure certain herpes virus infections in man) was inoculated into oral mucosa before, during, and after herpes simplex virus inoculation.

Major Findings:

During the first two days after virus inoculation, white plaques developed in the mucosal sites. These were followed by discrete, frank ulcerations which enlarged and coalesced so that at 5-6 days a single larger ulcer had formed. Signs of healing became evident at 7-9 days and epithelialization was usually complete within 9-12 days. From the second to the sixth day of their clinical course the ulcers gave positive cultures for herpes simplex virus, but after this period cultures were negative, although ulceration was still evident. Histologically, the viral lesions showed classical herpetic pathological alterations including ballooning degeneration of cytoplasm, chromatin margination, eosinophilic nuclear inclusion bodies, and giant cell formation in the oral epithelium.

Lesions produced by comparable doses of oral bacteria, or even much larger doses, were clearly different in character and pattern from the viral lesions. Bacterial lesions were confined primarily to the connective tissue, reached peak severity in two days, and produced only small ulceration overlying a superficial abscess. These small ulcerations had usually re-epithelialized within 3-4 days, whereas the viral lesions were undergoing enlargement at this time period. Although large inocula of oral bacteria and of human aphthous ulcer material failed to produce ulcers in the rabbit mucosa, large doses of Staphylococcus aureus and of orange peel extract did cause ulcers which were somewhat comparable in size to those caused by herpes simplex virus. Material taken from human aphthous ulcers at the most favorable clinical stages failed repeatedly to yield positive cultures for herpes simplex virus.

In the prophylaxis experiments, IDU had no effect on the development of herpetic mucosal ulcers, whereas previous exposure to the specific herpes simplex virus, either by single intramucosal inoculation or by

repeated parenteral inoculations, prevented mucosal ulceration. Anti-herpes antibody levels in the sera of protected rabbits ranged from < 1:256 to 1:16.

Significance to Dental Research:

The general significance of this work is that an in vivo test system has been applied to the study of oral ulcers, allowing many suspected ulcerogenic agents to be tested. Considerable experience has been gained using the rabbit oral mucosa as a test tissue, and a number of agents have already been tested for their ulcerogenic capacity. It is felt that proof of etiology for any agent or agents isolated from human aphthous ulcers must include a clear demonstration of ulcerogenic capacity in this or in a comparable animal system.

Of specific significance are the findings related to herpes simplex virus, human aphthous ulcers and bacteria. The production, for the first time, of experimental herpetic mucosal ulcers showing classical, clinical, and histological features and the recovery of the responsible organism from these lesions, satisfies two of Koch's postulates regarding the ulcers of human primary herpetic stomatitis. The failure to obtain herpes simplex virus from aphthous ulcers, even when cultures were taken at optimum stages, contributes further evidence that herpes simplex virus is probably not involved in the etiology of human aphthous ulcers. Further, the failure of aphthous ulcer material to produce ulcers when directly transmitted to rabbit mucosa suggests that aphthous ulcers may not be caused by a specific transmissible agent. The demonstration that acute local bacterial infections do not tend to ulcerate is interesting, but not surprising, and does not rule out bacteria as a possible etiological factor in aphthous ulcer formation.

Proposed Course of Project:

To perform additional acute and chronic tests of other microbial and nonmicrobial substances suspected of being involved in mucosal ulcer development. Such studies will involve chronic bacterial and mycotic local infections, and will also include tests of bacterial L forms which have been recently isolated from human oral ulcers.

Part B included No

Serial No. NIDR-13
1. Microbiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
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Part A

Project Title: Immunological, Biochemical, and Pathological Factors in the Taxonomy and Ecology of Oral Bacteria

Principal Investigator: Mr. Morrison Rogosa

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1962):

Total:	3-1/4
Professional:	1
Other:	2-1/4

Project Description:

Objectives:

To determine the immunological, biochemical and pathological characteristics of the veillonellae, lactobacilli, diphtheroids, and other oral microorganisms. This project is a continuation of NIDR-22 (1959).

Methods Employed:

1. Antisera, prepared in rabbits against bacterial cells, are tested against the organisms or antigens extracted from them by the techniques of agglutination, precipitation, and selective antibody absorption.

2. Biochemical characteristics are determined by standard and newly developed procedures.

3. Pathogenicity studies are conducted in appropriate animal hosts, inoculated by various routes.

Major Findings:

1. Taxonomic Studies. From the continuing studies since 1954, a great deal of definitive information now permits the clear differentiation of species of the genus Veillonella. Four papers are

being prepared: The genus Veillonella. I. General cultural, ecological and biochemical characteristics. II. Hydrogen sulfide production. III. Nutritional behavior. IV. Serological studies. Contrary to published descriptions, it has been shown that all strains, including type strains previously described by others, cannot utilize glucose or any other carbohydrate for energy, reduce nitrate, and produce hydrogen sulfide; and that the species Veillonella parvula, Veillonella alcalescens and their varieties possess distinctive noncrossing antigens. Also, hydrogen sulfide production has been shown to occur with cysteine, glutathione, thiocyanate, and thiosulfate but not with a large group of other sulfur compounds of different chemical structures. In cell-free systems a glutathione reductase enzyme was demonstrated. The nutritional experiments show that pyridoxal and thiamine are required by all strains, riboflavin by none, and that either putrescine or cadaverine are required absolutely by V. alcalescens. This amine requirement is different from that shown previously by Racker for certain Neisseria species since CO_2 does not obviate or minimize the specific amine requirement by V. alcalescens. The role of these amines in the metabolism of V. alcalescens requires further investigation. On the basis of the 4 manuscripts in preparation, V. parvula will be differentiated from V. alcalescens as follows.

(1) V. parvula: glucose negative; hydrogen sulfide and nitrite positive; putrescine or cadaverine requirement negative; catalase negative; oxidase and iron porphyrin negative; V. parvula antigen positive; and V. alcalescens antigen negative.

(2) V. alcalescens: glucose negative; hydrogen sulfide and nitrite positive; putrescine or cadaverine requirement positive; catalase positive; oxidase and iron porphyrin negative; V. alcalescens antigen positive, and V. parvula antigen negative.

Appropriate recommendations will be made to the International Committee on Nomenclature.

2. Biochemical Studies. Although the veillonellae grow well on lactate, resting cells metabolize lactate very poorly or not at all. Ammonium ion promoted the dissimilation of lactate by resting cells. This appears to be a hitherto unknown reaction and is being investigated. The expected intermediates in the fermentation of lactate, i.e., oxalacetate, pyruvate, malate, formate, and succinate, were all attacked readily with the formation of the expected products, i.e., propionate, acetate, carbon dioxide and hydrogen. Surprisingly, however, lactate and oxalacetate were metabolized aerobically with aerobic respiration by the anaerobic Veillonella. This phenomenon also is being investigated.

Significance to Dental Research:

Since systematic immunological, biochemical, and pathogenicity studies are lacking, these studies are important in differentiating organisms and their various physiologies and in determining the pathogenic potential of the oral microbiota.

Proposed Course of Project:

The immediate future will be devoted to further biochemical studies of the genus Veillonella. It is now clear that biochemical studies on V. alcalescens have not been previously done by anyone and that knowledge of the intermediate metabolism of this organism requires attention. Although the writer has recently been working hard on this problem, he strongly urges that physiologically oriented members of the laboratory be encouraged to cooperate in this very potentially fruitful field.

Part B included Yes

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Part B Honors, Awards, and Publications

Publications other than Abstracts from this Project:

None.

Honors and Awards Relating to this Project:

Member subcommittee on lactobacilli; secretary, subcommittee on Neissereaceae; member subcommittee on Bifidobacterium of the International Committee on Bacteriological Nomenclature. Life member, Washington Branch, Society of American Microbiologists.

Serial No. NIDR-14
1. Microbiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
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Part A

Project Title: Manifest and Inapparent Viral Infections of Oral Tissues

Principal Investigator: Dr. H. W. Scherp

Other Investigators: Mr. W. K. Ashe
Dr. R. J. Fitzgerald

Cooperating Units: None

Man Years (calendar year 1962):

Total:	3
Professional:	1-1/2
Other	1-1/2

Project Description:

Objectives:

1. To test for the presence of inapparent viruses in the tissues of "germfree" and conventional animals.

2. To isolate, identify, and determine the etiological significance of viruses from various lesions of the oral mucosa of undetermined etiology.

This project is a continuation of Project NIDR-2 (1961).

Methods Employed:

Samples containing tissue cells are frozen and thawed to liberate intracellular viruses. To test for viruses, the samples are inoculated into standard tissue cultures of rabbit kidney cells, HeLa cells, mouse L cells, and human skin cells. After incubation, cultures are frozen, thawed and transferred to a fresh tissue culture; serial transfers are made. In addition, specimens of biopsy tissue are maintained in tissue culture medium for protracted periods to allow development of latent viruses. Evidence for a virus is sought by microscopic examination for cytopathologic changes in the cultured cells and by hemagglutination and hemabsorption tests of the culture fluids with guinea pig, rabbit and chicken

erythrocytes. Isolates of viruses are identified by neutralization tests with specific antisera, when applicable. Refined antigenic analysis of related viruses is accomplished by comparison of the kinetics of their neutralization by homologous and heterologous antisera using a plaque-count method for quantitation of virus. Results are expressed as a pseudomonomolecular rate constant, K; homology is evidenced by the highest K values.

Major Findings:

1. Antigenic Analysis of Herpes Simplex Virus. It was reported in Project NIDR-2 (1961) that two strains of herpes simplex virus, isolated from the same individual during successive recurrences of herpes labialis two years apart, were immunologically distinguishable from each other although they were not distinguishable from other strains of the virus studied. On the basis of these findings a concerted effort was initiated to test the generality of this phenomenon and its significance to the pathogenesis of recurrent herpes labialis. The first step toward this end was to isolate strains of the virus during recurrent episodes of infection in a selected number of individuals. A total of 13 strains have been isolated from four individuals with known history of recurrent herpes labialis. Several of these strains were isolated at intervals approximating four years while others were isolated at intervals as short as two weeks. "Acute" and "convalescent" sera were obtained from each of these individuals during at least one of these recurrences in order to study the specificity of the antibody in these sera. Specific antisera against these strains were prepared in rabbits and the antibody titer of each of them was determined by routine serum neutralization procedures in order to obtain some idea of the range of serum dilutions to use in the K value experiments. Preliminary K value experiments have been completed for each serum and its homologous virus. These experiments have shown that a range of 1:20 to 1:80 dilution of serum can be used with 10^5 - $10^{5.8}$ plaque-forming units of virus to obtain optimal results. This range varies according to the particular serum employed. In order to study the immunological relationship between these recurrent isolations and the strains within the five serogroups which we have defined previously, a prototype strain from each of these serogroups was incorporated into the present study. Our data substantiate our previous finding that all of the strains of HSV isolated from the same individual during recurrent episodes of herpes labialis are not antigenically identical. Of five strains isolated from one individual over a period of 3-1/2 years, at least three are antigenically distinct from each other. The antigenic relationship between these strains and the prototype strains has yet to be determined.

An interesting but thus far unexplained observation was made in regard to four strains isolated from one individual over a five-month period. The first and second isolations, which were isolated exactly three

months apart, are antigenically identical to each other but antigenically different from the other two strains. The latter two strains were isolated from two lesions which occurred 13 days apart on different areas of the lower lip. However, they appear to be antigenically different. These four strains are of interest not only because they were isolated during recurrent episodes of herpes labialis, but also because they were isolated over such a relatively short span of time. Further study of the immunologic properties of these four strains is necessary before any definite conclusions can be drawn concerning their antigenic structure.

2. A Cytopathic Agent from the Salivary Glands of Rats. Previously we reported (Project NIDR-2 (1961) some of the chemical, physical and cultural characteristics of a possibly viral agent found in the salivary glands, but not in any of a variety of other organs and tissues of both conventional and germfree rats. During 1962, these properties have been studied in more detail.

Previously we found that we could transfer this agent serially in tissue cell culture only by using large vessels (Blake bottles) providing a large ratio of cell layer to inoculum. This method of passage slowed the development of the cytopathic effect so that discrete foci developed gradually. Presumably the net result was adequate opportunity for multiplication of the agent before it destroyed the host cells. In all, we have now carried the agent through 12 successive passages in two separate series, which is equivalent to at least a 10^{12} dilution of the primary inoculum. During these passages there was gross and microscopic evidence of amelioration and stabilization of the cytopathic effect and changes in its character, possibly indicative of adaptation or mutation of the agent. These results made possible a more detailed microscopic study of the development and nature of the cytopathic effect.

Cultures of rabbit kidney cells grown on cover slips were inoculated with the agent, then stained (May-Grunwald-Giemsa) at 24 hours, 72 hours, and 120 hours and examined microscopically. This facilitates a more precise analysis of the cytopathic effect of this agent. Preliminary observations have shown that in comparing inoculated cultures with controls, there appears to be a gross and intense eosinophilia involving the entire nucleus accompanied by some "mottling" of the nucleus. The nucleus also appears to be enlarged. Typical type A intranuclear inclusions are not seen although some cells seemed to have some atypical intranuclear inclusions characterized by disruption of the nucleoli without margination of chromatin. The cells appear to clump into masses which resemble in some respects syncitial formation. These effects become noticeable by 72 hours. By 120 hours, uninfected cultures show marked nonspecific degeneration but infected cultures do not show such degeneration. This

effect has been observed through five passages of the agent. Further passages are in progress to determine the extent and persistence of this phenomenon and to confirm these observations. Subsequently, attempts will be made to inhibit the cytopathic effects of this agent with specific immune serum prepared in rabbits.

Inoculation of the agent by various routes into suckling and young rats has resulted in no evident pathologic changes. Consequently, to determine its effect in other animals, newborn (24 hours old) mice and hamsters were inoculated. In both instances no pathologic effects were seen.

Undiluted rat salivary gland extracts and samples of cultures of the agent were passed through millipore filters, ultracentrifuged, and inoculated into rabbit kidney cell cultures. The agent passed through millipore filters (450 mμ) without loss of infectivity. Centrifugation for one hour at 105,000 G failed to sediment the agent completely, as evidenced by the presence of cytopathic activity in both the supernate and the sediment.

Studies to determine whether this agent is associated with a mycoplasma have been inconclusive and are being pursued, since it is essential to ascertain the presence or absence of this class of microorganism.

Significance to Dental Research:

Oral soft tissue lesions of unestablished but possibly viral etiology are frequently encountered by the dentist. The still incompletely understood phenomena of latency and activation of herpes virus are accordingly of direct dental interest; they also provide prototypes of fundamental viral relationships. The availability of a method to distinguish between strains of herpes virus will make possible elucidation of previously inaccessible phases of the ecology of this ubiquitous agent. Study of inapparent viral infection in otherwise germfree animals affords another approach to the study of the consequences of such infections on "normal" physiology.

Proposed Course of Project:

1. The cultural, physical and chemical properties of the cytopathic agent from salivary glands will be continued with emphasis on its immunological properties, determination of whether mycoplasmas are involved, further elucidation of particle size, and ultracentrifugation studies.

2. Investigation of the antigenic properties of strains of herpes simplex virus isolated from recurrent episodes of herpes labialis will be continued. The specificity of herpes antibody in human

serum obtained during and after the appearance of lesions will be studied using immunodiffusion techniques. The effect of high levels of such antibody on the antigenic structure of herpes simplex virus will be investigated by making use of viral carrier cultures and the kinetic serum neutralization technique.

Part B included Yes

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Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Burnett, G. W., and Scherp, H. W. Oral Microbiology and Infectious Disease. A Textbook for Students and Practitioners of Dentistry. Second edition. 1003 pp. The Williams and Wilkins Company, Baltimore, 1962.

Honors and Awards relating to this project:

None

Serial No. NIDR-15
1. Microbiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1962

Part A

Project Title: Nutrition and Metabolism of Lactic Acid Bacteria

Principal Investigator: Dr. T. Shiota

Other Investigators: None

Cooperating Unit: None

Man Years (calendar year 1962):

Total:	3
Professional:	1
Others:	2

Project Description:

Objectives:

To study amino acid, peptide and vitamin metabolism of lactic acid bacteria with specific attention to the enzymatic synthesis of folic acid-like compounds.

Methods Employed:

1. The determination of folic acid-like compounds was carried out by microbiological assays, chromatography and spectrophotometry.

2. Phosphorylated pteridines were characterized by phosphorus and nitrogen analyses, electrometric titration and by treatment with specific enzymes, such as phosphomonoesterase and pyrophosphatase.

3. The purification of the folate-synthesizing enzyme system was performed by column cellulose chromatography and ammonium sulfate fractionation.

Major Findings:

The dihydrofolate (DHFA) synthesizing enzyme from Lactobacillus plantarum was previously shown to couple a chemically prepared dihydropteridine containing two phosphates (Pt-PP) to p-amino-benzoylglutamate (PABG). It was proposed at that time that this

phosphorylated compound was the dihydro-derivative of 2-amino-4-hydroxy-6-pteridylmethyl-pyrophosphate. The proposed reaction is as follows:

$$\text{Pt-PP} + \text{PABG} \longrightarrow \text{DHFA} + \text{PP (pyrophosphate)}.$$

First, various experiments were carried out to characterize this phosphorylated pteridine. From electrometric titration data, acid and enzyme hydrolysis and spectrophotometry, we concluded that the identity of this compound is as proposed, the pyrophosphate derivative of the pteridine. Second, the validity of the proposed reaction is being tested by demonstrating the stoichiometry of the reaction. Assay procedures, such as the purification of the enzyme system and the development of a micro-method of pyrophosphate and p-amino-benzoylglutamate, are being worked out.

Significance to Dental Research:

Since many microorganisms in the oral cavity are fastidious, this would imply that essential nutrients are therefore present. The presence of these essential metabolites necessary for the growth of microorganisms can be accounted for, in part, by the biosynthetic capacity of the microbiota. Therefore, the study of how these metabolites are formed may help in the understanding of the relationship between the occurrence of certain microorganisms and oral health problems.

Proposed Course of the Project:

It is proposed that the project on the biosynthesis of folic acid-like compounds be continued. (1) We hope to purify the enzyme system of L. plantarum so that we can demonstrate a pyrophosphokinase for the reduced pteridine. (2) The biosynthesis of peptide forms of folate compounds has not been adequately studied; therefore, we have initiated investigations of this problem. (3) Studies leading to other possible coenzyme activities of these reduced pteridines are also being explored.

Part B included Yes

PHS-NIH
Individual Project Report
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Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Shiota, T., Disraely, M. N., and McCann, M. P. Preparation of Dihydropteridinediphosphate, an Intermediate in Dihydrofolate Synthesis. Biochemical and Biophysical Research Communications 7: 194-198, 1962.

Shiota, T., Blumenthal, H., Disraely, M. N., and McCann, M. P. Tagatose 6-Phosphate Formation from Galactosamine 6-Phosphate by Cell-Free Extracts of Lactobacillus casei. Arch. Biochem. and Biophys. 96: 143-146, 1962.

Honors and Awards relating to this project:

None

Serial No. NIDR-16
1. Microbiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1962

Part A

Project Title: Studies on Lactate Fermentation by Butyribacterium rettgeri.

Principal Investigator: Dr. C. L. Wittenberger

Other Investigators: None

Cooperating Units: American Dental Association

Man Years (calendar year 1962):

Total:	2
Professional:	1
Other:	1

Project Description:

Objectives:

This is a continuation of Project NIDR-8 (1961). The anaerobic bacterium Butyribacterium rettgeri carries out a modified butyric acid type fermentation of lactate. The major products of the fermentation are butyrate, acetate and carbon dioxide. An unusual feature of this metabolic process emerged when Barker and co-workers discovered that the organism required lipoic acid for growth when lactate served as the main energy source but not when pyruvate or glucose was substituted for lactate in the growth medium. These results suggested that lipoic acid was not involved in the classical α-keto acid oxidation sequences but rather was functioning in a new and perhaps unique metabolic capacity. Other studies by Barker's group on the metabolism of B. rettgeri revealed that the acetate formed by this organism is partially derived from the condensation of two moles of carbon dioxide. The nature of the pathway and the intermediates involved in this fixation of CO_2 has not been resolved.

This investigation is oriented toward resolving the role of lipoic acid in lactate dissimilation by B. rettgeri and toward determining the pathway of acetate formation from carbon dioxide. It is hoped that the information derived from this study will not only provide a basis for a better understanding of the metabolism of the organism but might also, in a broader sense, contribute to the general

knowledge of carbon dioxide fixation processes and the metabolism of one-carbon units.

Methods Employed:

All are standard techniques routine to the types of studies herein described.

Major Findings:

Previous studies from this laboratory had shown that in the fermentation of lactate by B. rettgeri, lipoid acid functions as an integral component of the electron transport system involved in the disposition of lactate hydrogens. The vitamin does not participate in the fermentation of pyruvate by B. rettgeri. The experimental approach to the problem of this unusual role of lipoate has been largely dictated by the lability of some mediating component of the process. Cell-free extracts, prepared by a wide variety of techniques, are completely unable to couple lactate dissimilation to the reduction of lipoic acid, although this activity is easily demonstrable in resting cell suspensions of the organism. In an effort to gain additional information concerning the metabolic role of lipoic acid in the lactate fermentation, studies have been initiated on the metabolic capabilities of a culture of B. rettgeri maintained on glucose medium without lipoate (strain G). As indicated before, B. rettgeri can be grown in a glucose synthetic medium devoid of lipoic acid. It was felt that an analysis of the metabolic potentialities of this glucose strain might provide additional information about certain metabolic sequences in the strain (L), which is maintained on lactate medium with lipoate. For example, the absence of an enzyme or enzyme system in the glucose strain might reflect an involvement of that enzyme or enzyme system in the lactate strain.

B. rettgeri G has been shown to ferment glucose to acetate, butyrate, and CO_2. Identical products are formed during the fermentation of lactate by B. rettgeri L. In addition, B. rettgeri G incorporates CO_2 into both the carboxyl and methyl carbons of acetate just as does B. rettgeri L. It is concluded that lipoic acid, therefore, is not obligatorily involved in these metabolic sequences. The only major metabolic difference detected between the G and L strains of the organism was that strain G accumulated lactate as one of the major glucose fermentation products. Resting cell suspensions of B. rettgeri G have been found to be unable to carry out the anaerobic decomposition of lactate.

Cell-free extracts from B. rettgeri G contain an active DPN-linked lactic dehydrogenase. This enzyme has been purified about ten-fold and some of its properties have been studied. The presence of this

enzyme in B. rettgeri L is equivocal. Either the enzyme is present in much smaller amounts in this organism or it is present in a more labile form. On the other hand, cell-free extracts from B. rettgeri L possess an enzyme system capable of coupling the anerobic dissimilation of lactate to the reduction of ferricyanide or 2,6-dichlorophenolindophenol. This activity is absent in extracts of B. rettgeri G. These observations and others have led to the tentative conclusion that the DPN-linked lactic dehydrogenase functions primarily in the formation of lactate from pyruvate, whereas the ferricyanide reaction reflects an enzyme system primarily involved in the dissimilation of lactate.

The biochemical pathway by which B. rettgeri incorporates CO_2 into both the methyl and carboxyl carbons of acetate is also under investigation. This unusual reaction has been demonstrated in several other anaerobes including Clostridium aceticum and Clostridium thermoaceticum. However, virtually nothing is known concerning the intermediate steps involved in this metabolic pathway. Studies on the CO_2-fixation process in B. rettgeri have been hampered by the inability to obtain cell-free extracts of the organism which were capable of effecting this reaction. Recently it has been found that extracts prepared from a glucose strain of B. rettgeri do incorporate CO_2 into acetate. The activity is quite low compared to that observed with resting cell suspensions. The reaction in extracts shows a pH optimum of about 6.4 and is dependent upon phosphate and a source of reducing equivalents. Glucose has been used as the source of reducing equivalents. Glucose cannot be replaced by substrate levels of DPNH, TPNH or dihydrolipoic acid and therefore probably provides energy as well as reducing equivalents. In crude extracts, the reaction appears to be stimulated by biotin and possibly tetrahydrofolic acid but is not affected by vitamin B_{12}, coenzyme A, cocarboxylase and a variety of other cofactors.

Significance to Dental Research:

Many of the organisms comprising the oral flora, such as Veillonella, require rather high partial pressures of carbon dioxide for growth. Indeed, so far as known, virtually all living organisms require a certain level of carbon dioxide for growth and reproduction. The nature of this carbon dioxide requirement is incompletely understood and it is anticipated that studies on the mechanisms of carbon dioxide fixation in B. rettgeri will provide basic information which will contribute to the better understanding of this fundamental phenomenon in all organisms. Similar considerations apply to study of the functions of lipoic acid.

Proposed Course of Project:

Studies now in progress are oriented toward ascertaining why

B. rettgeri G does not possess an enzyme system for decomposing lactic acid. Answers to the following questions will be sought:

1. Is the lactate decomposing system adaptive and its formation repressed by glucose?
2. Is the glucose strain of B. rettgeri actually a mutant of the parental L strain which is blocked in its ability to form an enzyme or enzyme system necessary for lactate dissimilation?

A continuation of the studies on the CO_2 fixation process in B. rettgeri is anticipated. Efforts will be made to establish the nature and number of intermediate compounds involved in this biochemical sequence. A detailed evaluation of the possible role of biotin and tetrahydrofolic acid in the CO_2 fixation process will be undertaken.

Part B included No

HISTOLOGY & PATHOLOGY

17. D. B. Scott: Studies of the Structural, Physical, and Chemical Properties of Calcified Tissues by Electron Microscopy and Electron Diffraction.

18. K. A. Omnell: Microradiographic and Histochemical Studies of Tooth Mineralization.

19. M. U. Nylen: Crystal Growth in Rat Enamel.

20. H. Moe: Studies of the Cytodifferentiation of Odontoblasts and Ameloblasts.

21. K. A. Selvig: Ultrastructural Studies of Dental Cementum.

22. H. A. Bladen: Studies Concerning the Ultrastructure of Bacteroides: A Genus of Bacteria Found in the Oral Cavity.

23. M. U. Nylen, D. B. Scott, and H. A. Bladen: Secondary Collaborative Projects and Training Activities.

24. A. S. Posner: Crystal Chemistry of Calcified Tissue and Related Compounds.

25. A. S. Posner: Infrared Absorption Spectrophotometric Studies on Calcified Tissues and Related Synthetic and Natural Compounds.

26. H. M. Fullmer: Histochemical Studies of Connective Tissues.

27. P. H. Keyes: Experimental Dental Caries.

28. P. H. Keyes: Experimental Periodontal Disease.

Annual Report of the Laboratory of Histology and Pathology
National Institute of Dental Research
Summary Statement

In prior years the Summary Statement of the activities of the Laboratory of Histology and Pathology has taken the form of a descriptive catalogue of research accomplishments. During the current year certain marked changes in the attitudes and ambitions of key members of the Laboratory staff have become evident which make it advisable to include additionally within the narrative report an assessment of program activities in terms of present scope, long-range potential, and practical steps that might be taken to meet immediate needs. The above mentioned reactions are not to be interpreted as reflecting dissatisfaction on the part of the staff, but on the contrary bespeak a realization that the research problems that have hitherto been considered as independent investigations have now reached proportions that merit extension into broader programs. As background information, the original nucleus of this Laboratory was a small group working with physical methods, primarily electron microscopy and diffraction, on the structure and properties of the calcified tissues. Activities were subsequently added in histochemistry, mainly directed toward the study of connective tissues, experimental pathology, dealing primarily with experimental dental caries, and crystal chemistry, concerned with atomic structure of tooth and bone mineral. The total professional complement has always been small, and interestingly enough, almost every individual has been inclined toward conservatism regarding expansion, preferring to wait for the time when justification seemed adequate. It is not surprising that the desire for expansion in all four areas should be expressed simultaneously, for the responsible investigators are all senior scientists, whose careers have run more or less in parallel. These individuals have by now gained international reputations, and their maturity in research is well established. Perhaps the most influential factor in the present situation, aside from the previously stated sharpening of research focus, has been the markedly improved working conditions and facilities over the past year.

The fact that the research of the past year has been most fruitful is attested to by the fact that the professional Laboratory staff, consisting of seven permanent members and six visiting scientists (all of whom were not on duty for the full year), contributed to the production of 37 publications. On 26 of these they assumed first authorship. This statistic and a recognition of the varied subject material covered, bear out the facts that there is no dearth of ideas among these scientists, and that to expect productivity beyond this without some provision for expansion would be unreasonable. A start in this direction has been made within the last month by the establishment of a Section on Crystal Chemistry, the implications of which will be discussed below.

At this point note should be made that recruiting, even on the small scale possible over the past several years, has presented serious problems, especially in the area of biophysical scientists. Competition from industry and universities has proved overwhelming. This is demonstrated by the fact

that for one reason or another of 22 individuals contacted in the past two years, none were recruited. Admittedly, some of these did not prove to be desirable candidates. The fact that this unavailability of permanent personnel cannot be accounted for by scientific or physical unattractiveness of NIH is evident, because it has been easy for this Laboratory to recruit Visiting Scientists of the highest order of competence, as well as trainees (Special Fellows, Guest Workers, and Research Associates). Fortuitous as this latter circumstance may be for the perpetuation of the Laboratory program, it will be essential to improve our recruiting system. The problems may not be immediately soluble, however, inasmuch as the primary considerations appear to be either salary or general lack of individuals qualified in the research areas in question. It may be necessary to resort further to recruiting individuals who have biological or dental backgrounds and are amenable to specialized on the job training. This has worked very successfully in two cases and might provide a partial answer to the personnel problem.

With the preceding discussion in mind, the following summary of research accomplishments, together with projected needs is presented according to the subdivisions into which the activities of this Laboratory may be classified:

1. Biophysical Research. In this area the projects have primarily consisted of continued basic studies of the ultrastructure of various calcified tissues, dental embryology, and mineralization mechanisms, as well as new investigations in cytology and microbiology. As would be expected in research involving physical methods such as specialized light microscopy, electron microscopy and diffraction, contact and projection microradiography, densitometry, etc., considerable time and effort must be spent in technical and instrumental development. This year, for example, the studies on crystal development became so exacting that improvement of embedding methods and precise orientation of specimens for thin sectioning was essential. In addition, a rather extensive survey of the properties and usefulness of numerous newly suggested embedding media had to be made. A major effort concerned the development of adequate techniques for contact and projection microradiography, as well as modification of commercially manufactured equipment so that it could meet the experimental demands. As matters now stand many of the difficulties in specimen preparation have been solved, cameras of various types have been constructed and are in regular service, and the projection X-ray microscope has been modified so that specimen accommodation, focusing, camera inadequacies, magnification calibration and several other inherent limitations no longer present problems. Microradiography has by now reached the dimensions of a specialized field, the coming aspect of which is microanalysis.

   Work on the structure and properties of mature human tooth enamel has continued, and a good start has been made in explaining through electron microscopic studies various of the hitherto unclarified morphological configurations long observed under the light microscope.

Perhaps the most important findings concern the striation patterns in the enamel prisms, which have previously had several different interpretations, and which have structural implications in the progression of dental caries. Apparently these rhythmic striations do not reflect differences in the ratio between organic and inorganic components, but may be accounted for in terms of regular variations in microcrystalline orientation which produce summatively the observed optical effects. In other studies on mature enamel, the surface alterations and changes in solubility effected by stannous fluoride, especially as compounded in dentifrices, have been explored further. A principal finding has been the surprising degree of *in vitro* protection against acid action provided by very low levels of available stannous fluoride.

Several observations of major importance have come from the studies of enamel development. In microradiographic examinations aimed at defining the calcification sequence through density changes a disturbing physical effect was found which indicates the need for reassessing the validity of essentially all of the recently reported X-ray data. It was discovered that X-ray absorption is much higher when the radiation passes through the enamel crystals parallel to their c-axes than when it passes through perpendicularly. The explanation for this phenomenon is still being sought, but its implications insofar as quantitative estimations of the degree of mineralization in enamel, where crystal orientation varies considerably from area to area, are self-evident. On the positive side, some excellent data have been obtained regarding crystal growth in enamel, and on the structural interrelationships between the organic and inorganic components. The work on crystal growth has required high resolution electron microscopy of precisely prepared sections containing cross or longitudinally cut crystals. In combination with electron and X-ray diffraction studies, the data show clearly that there is very rapid growth in length (c-axis) following nucleation in the newly formed organic matrix at the immediate ends of the formative cells, and then a slow and steady growth in width and thickness until the final hexagonal cross sectional contours are developed. Observation of parallel lines within the tiny crystals, which are in fact reflections from atomic intracrystalline planes, indicate a degree of structural perfection that essentially rules out the presence of organic matter within crystals. Further progress, on the other hand, has been made in defining more precisely the distribution of organic matrix between and immediately around the apatite crystals. The importance of exact characterization of the inorganic-organic relationships in enamel is in the fact that herein may lie one of the most important keys to an understanding of how the enamel disintegrates in the caries process.

Inasmuch as the origins of all the calcified tissues are basically cellular, work has been continued on the ultrastructure of the cells involved in enamel, dentin and cementum formation. Aside from the purely anatomical connotation of such studies, the major points to be settled concern exactly what any of these cells have

to do with the elaboration of the organic matrices, and the possible role they play in the calcification process. Other aspects in cytology which will be given attention are the development of adequate methods of tissue handling and staining procedures, so that histochemical investigations can be made at the electron microscopic level, and observations on the structure, properties and embryonic sequences of cementum.

Through the training of a microbiologist over the past year, it has been possible to extend collaborative studies on bacterial mineralization, as well as initiate ultrastructural investigations of certain oral microorganisms. The latter project, concerned mainly with the genus Bacteroides, has led to the observation of what may be conjugatory bridges. Although the phenomenon of conjugation, a process by which genetic characteristics are transferred between bacteria, has been known to occur for a long time, it seems to be a very rare event. If the present interpretation of the electron microscopic data should prove correct, it is the first time visual evidence of actual interconnections between microorganisms has been recorded.

Other collaborative projects with workers in laboratories within and outside NIH have dealt with aspects of the mineralization process, and cytological, microbial and viral problems.

2. Crystal Chemistry. Concentrated work in this area was begun a year and a half ago with the establishment of a program for the conduct of crystallographic studies. Here the major approaches are made through X-ray diffraction in all its forms, and through infrared spectrophotometry. By these techniques investigations are being made of the crystal texture of hard tissue apatites and synthetic calcium phosphates of similar composition, in which crystal size and strain are under study first from the standpoint of pure basic definition, and second from the standpoint of structural improvement that may be achieved by various induced chemical alterations. Single crystal X-ray analyses are also being conducted for the purpose of elucidating the exact atomic arrangements within crystals of bone and tooth mineral, as well as the substitution sites of various ions and groups in both natural and synthetic apatites. Coupled with these studies, hydrogen bonding in the calcium phosphates in general is being investigated by infrared absorption spectrophotometry. The basic aim here is to gain an understanding of the manner in which electrical neutrality is maintained in structures which prove to have less than the stoichiometrically required number of calcium atoms. It is thought that this is achieved through the presence of hydrogen bonds between orthophosphate oxygens. The significance of this phenomenon lies in the effect it has on the stability and solubility of bone and tooth mineral.

During the past year it was found that fluoride and hydroxyl ion occupy identical structural positions in hydroxyapatite when substituted for each other, while chloride ion assumes a different position. A most important observation was made with respect to the effect of fluoride ingestion in humans and laboratory rats on the crystalline character of bone mineral. The X-ray studies have shown that with increased fluoride content the bone apatite crystals tend to be larger and more perfect structurally, while the infrared observations have demonstrated further a crystal perfection through reduced calcium deficiency. Thus, for the first time some concrete evidence has been obtained as to the exact way in which fluoride acts upon biological mineral to make it more stable, by increasing crystallinity and by the production of a less soluble fluoride-substituted apatite. The continuation of these initial studies will be a refined investigation of the role of fluoride in synthetic apatite crystal nucleation and growth, followed by a reapplication of the findings to other biological tissues, especially tooth enamel. In addition, the changes in stoichiometry of biological apatites with age, disease and dietary changes will be important to determine as background for experiments with not only fluoride compounds, but also other possible media for effecting improved mineralogical status.

Emphasis in the immediate future will be placed on crystal texture in organized tissues and on single crystal structure. This work will be extended to include crystal nucleation and growth, and important studies will be undertaken on the organic components of the calcified tissues. One of the most immediate needs in this latter area is to characterize the chemical nature of the organic matrix of enamel. This cannot be done by chemical analysis, and so far there remains considerable dispute as to the real nature of the fibrous keratin-like protein in enamel. To extend this program it is planned to recruit at least two more physical chemists or physicists, and to set up facilities for synthesis of the larger amounts of experimental compounds required. As might be expected, much of the work will continue to be performed in collaboration with other workers outside the section, who have specialized knowledge either in allied instrumentation or in the handling of biological specimens.

3. Histochemistry and Experimental Pathology. Studies in this area have continued to concentrate on the chemical composition and reactive groups in normal and diseased connective tissues. The significance of the oxytalan fibers discovered here in 1958 has become more clear, and it is now thought that they belong to the elastic family of connective tissue fibers. There is an implication in this year's results that they may actually be a precursor of elastic fibers.

In another histochemical study of periodontal disease in human autopsy material, it has been found that degradation of connective tissue and bone may occur at sites distant from the inflammatory

focus. This suggests a weakening of periodontal structures in advance of any actual inflammatory process. Data such as this provide another link in the search for sequential processes involved in periodontal disease, and it is hoped that through the histochemical approach some of the mechanisms will be clarified.

Stemming from the observation several years ago of transmissibility of dental caries in animals, it became obvious that serious attention should be given to the microbial factor. This year the cariogenic potential of several new bacterial isolates, primarily streptococci, has been tested, and numerous agents assayed for their effectiveness in reducing the cariogenic microflora. Improvements have also been made in test diets which have provided for more normal growth in animals, as well as a marked enhancement in producable caries activity. The important feature of all these studies is that the experimental conditions have been carefully defined and rigorously controlled, so that more specific information could be obtained.

In parallel with the studies on caries, some evidence has now been assembled to suggest that periodontal lesions can be transmitted from diseased to normal animals through the introduction of scrapings of subgingival plaque material from the strain of animals in which the disease had developed spontaneously. Thus, there has begun a chain of experiments which can be carried on much in the same fashion as those dealing with caries. The pathogenicity of certain filamentous microorganisms has been established this year by introducing and recovering strains previously labelled by an induced resistance to the antibiotic, terramycin. The development of a laboratory technique for inducing and studying periodontal disease has aroused considerable interest, and it is more than likely that extension of this project will be inevitable.

Serial No. NIDR-17
1. Histology & Pathology
2. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Studies of the Structural, Physical, and Chemical Properties of Calcified Tissues by Electron Microscopy and Electron Diffraction.

Principal Investigator: Dr. D. B. Scott

Other Investigators: Dr. R. G. Hewes (special consultant)

Cooperating Units: None

Man Years (calendar year 1962):
Total: 3 1/4
Professional: 1
Other: 2 1/4

Project Description:

Objectives:

1. Determination of the crystal structure of human enamel, with special emphasis on crystal orientation and size in the mature tissue.

2. Investigation of chemical and physical reactions of fluoride compounds on tooth structure.

3. Development and standardization of instruments and techniques for contact and projection microradiography as applied to mineralized tissues.

Methods Employed:

For studies of the crystalline organization of mature enamel the most productive results have come from the study by electron microscopy and electron diffraction of positive real and pseudo-replicas made from chemically treated surfaces of ground tooth sections, combined with observation of the original specimens or counterpart preparations by optical microscopy, using transmitted or incident ordinary light as well as polarized light.

Various methods of treatment with fluoride compounds are employed and the effects on enamel are largely being assessed on the basis of solubility changes as observed by gross, optical or electron microscopic examination.

Microradiographic technique has presented a number of problems, which have had to be solved before the full potential of this new area of instrumentation can be realized. So far as contact methods are concerned, it has been necessary to explore in some detail the inherent properties of our various available sources of radiation, the intrinsic requirements of specimens which are adequate for examination, and the construction of special cameras and associated devices which meet the needs established by the present experimental conditions. With projection microradiography the problems have been somewhat different. Here it has been necessary to experiment with the wide variety of readily interchangeable X-ray target materials which can produce a range of useful monochromatic radiation wavelengths from roughly 1.5 to 10 Å, as well as to explore the types of specimens which can be examined, and further to develop and evaluate instrumental modifications to overcome basic commercial design limitations associated with specimen stage mechanisms, instrumental focus, camera size, and stereomicroscopy. Darkroom procedures for presentation of morphologic detail have also been developed by logetronic printing techniques.

Major Findings:

1. Those phases of the studies on the crystalline organization of mature human enamel which could be profitably carried out with the available techniques were largely completed during the preceding calendar year, and in prior work with Professor J. G. Helmcke, a Visiting Scientist from the Fritz-Haber Institute in Berlin. Review of the collective data and the arrival at an agreement between the investigators involved regarding interpretation have consumed considerable time during this year, and a meeting of minds has been reached. Without going into undue detail, the forthcoming publication deals with data regarding the distribution pattern of apatite crystals within the enamel prisms, and with a possible explanation of the rhythmic striation patterns always observed in ground sections under visible light. Contrary to prior ideas about periodic quantitative differences in organic content, the present conception is that the striations reflect a summative optical effect produced by superimposed layers of crystals in specific angular orientation with respect to the rhythmically increasing and decreasing diameter of each prism over its entire length.

2. Work on the reaction of fluoride compounds on enamel has been concentrated on the effect of SnF_2 when applied in the presence of a dentifrice formulation. The project has been predicated on two bases, the first being that it is a natural outcome of an extensive series of prior studies of the mode of action of aqueous solutions in bringing about a marked reduction in solubility. The second basis was a request for assistance from the Division of Dental Public Health and Resources for information on a commercially produced standard dentifrice which is being specially packaged for a field study on the efficacy of a stannous fluoride dentifrice as an adjunct in public health dental programs. The primary question asked concerned comparability of the product with that obtainable on the open market. So far as this Laboratory was concerned, it was of interest to obtain this specimen material, which has consisted of both experimental and control (non SnF_2) dentifrices from the actual study and samples obtained by open marked purchase. Of particular value has been the freshness of the experimental product. It has been possible to extend the studies of solubility reactions in this fashion so that a number of interesting data have been forthcoming. Perhaps the most important of these is that regarding the apparent low level of actually available SnF_2 in a dentifrice (due to internal reaction, hydrolysis and complexing) which is still capable of producing a profound in vitro effect on enamel solubility. Various other properties of SnF_2 as well as NaF when compounded with other substances have become more clear, and an insight into directions for future fluoride research is being gained. It should be emphasized that such data are still basic, and any clinical inferences would be unwarranted.

3. The advancement of microradiographic technique has been one of the major efforts in this project. Without going into a description of the trials and negative aspects of technical development, the present status is as follows. Modifications have been made (1) to quadruple the area which can be exposed to radiation in the Philips CMR apparatus, (2) the limitations of the radiation provided by this 5KV source have been defined and specifications for adequate specimens have been established, (3) a special camera readily interchangeable on the track of a standard high voltage X-ray generator has been built for use with thick, dense samples, (4) a vacuum specimen holder for use in the latter camera has been developed, and the required characteristics of specimens determined, (5) the focusing and aperturing problems associated with the Norelco projection X-ray microscope have been solved, (6) specimen stage and camera modifications, and a magnification monitor have been built for the latter instrument, (7) specimen characteristics for projection microscopy, both plane and stereo,

have been better defined, and the required high tensions and X-ray target materials have been explored. In addition, applications of these various procedures have been made in many of the projects in progress in the Laboratory, these being referred to in the appropriate reports.

Significance to Dental Research:

These continuing experiments are directed toward developing through biophysical methods data required for an understanding of the structure and composition of normal tooth substance and its reaction to varied physical and chemical influences.

Proposed Course of Project:

Continued refinement of ultrastructural information on enamel and dentin, as well as ultimate resumption of studies on the alterations in pathological conditions, primarily caries. In addition, further exploration on mechanisms of possible agents proposed for caries control, such as fluoride compounds.

Part B included: Yes

Serial No. NIDR-17
1. Histology & Pathology
2. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1962

Part B: Honors, Awards, and Publications.

Publications other than abstracts from this project.

Scott, D. B., and O'Neil, J. R. The Microstructure of Enamel and Dentin as Related to Cavity Preparation, in Adhesive Restorative Dental Materials, R. W. Phillips, Ed., Owen Litho. Spencer, Indiana, pp. 27-37.

Scott, D. B. Biophysics in Dental Research, in Vistas in Dental Science, Ed., Nat. Ass. Sci. Teachers, (In press).

Scott, D. B. Laboratory Investigation of Fragmentary Medicolegal Specimens (In press).

Arnold, F. A., Likins, R. C., Russell, A. L., and Scott, D. B. Fifteenth Year of the Grand Rapids Fluoridation Study, in "Fluoride Drinking Water," F. J. McClure, Ed., PHS Public #825, U.S. Gov't. Printing Office, 1962, pp. 253-256.

Scott, D. B., Nylen, M. U., and Pugh, M. H. Some Technical Aspects of Microradiography. Norelco Reporter 9:102-109, 1962.

Scott, D. B. Microradiographic Surveys as a Preliminary to Electron Microscopy. Encyc. of X- and Gamma Radiation (in press).

Helmcke, J. G., Schulz, L., and Scott, D. B. Cross-striations in Enamel Prisms (in press).

Honors and Awards relating to this project:

None

Serial No. NIDR-18
1. Histology & Pathology
2. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1962

Part A.

Project Title: Microradiographic and Histochemical Studies of Tooth Mineralization.

Principal Investigator: Dr. Karl-Åke Omnell

Other Investigators: Dr. Marie U. Nylen

Cooperating Units: None

Man Years (calendar year 1962):
Total: 5/8
Professional: 1/4
Other: 3/8

Project Description:

Objectives:

Previous microradiographic studies of developing enamel from humans, monkeys, dogs, and cats have shown striking similarities in the gross pattern of mineralization. Preliminary microradiographic investigations in this laboratory indicated, however, that the gross pattern of rat enamel mineralization differed from that observed in the above mentioned species. The aim of the present project was to extend the preliminary investigations and to relate the microradiographic image to the submicroscopic structure of the enamel examined. Since it has been demonstrated that a proteolytic enzyme (aminopeptidase) is present in developing enamel it was further decided to determine the stage of development with respect to degree of mineralization at which this enzymatic activity can be found.

Methods Employed:

The upper incisors from 75 day old rats and developing rat molars were dissected out, fixed in neutral formalin, and embedded in Bio-Plastic. Twenty to one hundred micron thick ground sections were prepared. Contact microradiographs were obtained using Ni-filtered Cu K radiation from an X-ray diffraction generator (35 Kv)

or a continuous radiation with a longer wavelength from a Philips CMR unit (5 Kv). For electron microscopy small enamel pieces were dissected from the ground sections, embedded in methacrylate, sectioned with diamond knives, and examined in the Siemens Elmiskop I. For histochemistry developing molars were frozen and sectioned in a cryostat. The sections were subsequently incubated in a medium prescribed by Nachlas, Crawford and Seligman.

Major Findings:

Enamel formation began with the elaboration of a considerable amount of enamel which showed very little density difference between that close to the dentino-enamel junction and the outside layer. The presence of mineral crystals was, however, shown with the electron microscope, and it was this partly mineralized enamel which stained uniformly reddish pink, indicating aminopeptidase activity. Following this initial stage differences in absorption became apparent, but instead of a gradient in density from the junction outwards, the highest absorption was obtained about midway between the dentin and the enamel surface. Subsequently, a band formed along the dentino-enamel junction, and the two bands gradually expanded toward each other and toward the enamel surface. At the final stage the two zones merged completely, and the enamel appeared to have a rather uniform density throughout. Apart from the gross pattern of absorption changes just outlined, alternating radiopaque and radiolucent stripes were observed extending from the dentino-enamel junction toward the enamel surface. The direction and thickness of the lines corresponded to the direction and thickness of the enamel rods. The interpretation of the lines as images of enamel rods was confirmed by electron microscopic studies of identical regions, and it appeared that the difference in absorption was due entirely to differences in rod and consequently crystal orientation from one layer to the next. Thus the X-ray absorption was lower when the X-rays penetrated a given volume of enamel perpendicular to the c-axis than when they penetrated the same volume parallel to the c-axis. With the finding of a relationship between crystal orientation and X-ray absorption the entire study will have to be extended before any conclusions can be reached regarding a gross pattern of mineralization.

Significance to Dental Research:

Our present day knowledge and understanding of the mineralization process in enamel has to a large extent been based on microradiographic studies. All of these investigations will have to be reevaluated in the light of the present observation that differences in X-ray absorption may be caused by differences in crystal orientation rather than by actual variations in degree of mineralization.

Proposed Course of the Project:

The absorption phenomenon observed in enamel containing well aligned crystallites will be further investigated using qualitative as well as quantitative microradiography.

Part B. included: No

Serial No. NIDR-19
1. Histology & Pathology
2. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1962

Part A.

Project Title: Crystal Growth in Rat Enamel.

Principal Investigator: Dr. M. U. Nylen

Other Investigators: Dr. K.-Å. Omnell and Dr. E. D. Eanes

Cooperating Units: None

Man Years (calendar year 1962):

Total:	1 7/8
Professional:	1/2
Other:	1 3/8

Project Description:

Objectives:

The degree of mineralization can be determined in a number of ways, notably by chemical analysis and by microradiography. A study of rat enamel mineralization utilizing the latter method resulted, however, in some very controversial data, and a need was felt for the development of a new approach to the problem. The aim of the present work has been to study developing enamel and especially the changes in number, size and shape of the crystals which occur during maturation, in the hope that a set of standards can be developed which will permit estimation of the degree of mineralization within small areas on the basis of electron microscopic observations.

Methods Employed:

Small pieces of enamel were dissected from ground sections of developing first molars and upper incisors of 75 day old rats. Some of the pieces were embedded in butyl/methyl methacrylate and thin sections were cut on an LKB Ultrotome equipped with diamond knives. The most instances the thin sections were examined directly in the Siemens Elmiskop I. Occasionally they were decalcified first by floating them for one hour on a 2% phosphotungstic acid solution. Other enamel pieces were mounted intact on glass fibers

which were placed in a Debye-Sherrer type powder camera. X-ray diffraction photographs were taken with Ni-filtered Cu Ka radiation.

Major Findings:

Crystal nucleation appeared to take place in the immediate vicinity of the ameloblasts, and as maturation progressed the crystals gradually increased in size. The striking feature, however, was that within a given area or layer all the crystals were of nearly the same size, indicating that progression of mineralization depended entirely on crystal growth and not on the formation of additional crystals. This finding made it possible to use the size of the crystals, as an indicator for the relative degree of mineralization. The earliest crystals took the shape of extremely thin, long plates arranged in such a way that there seemed to be little room for any further lengthwise growth. With advancing mineralization, on the other hand, the crystals very gradually increased in thickness and assumed the shape of long, hexagonal rods. Once the hexagonal shape was established the crystals seemed to enlarge at the same rate in both width and thickness. While the youngest crystals observed were approximately 300A wide and 10A thick, the most mature crystals measured 500-600A in width and 250-300A in thickness. The growth pattern deduced from the X-ray diffraction studies corresponded to that observed in the electron microscope, i.e. the crystals were found to be very long in the c-axis direction at an early stage of development. While the X-ray method employed was not refined enough to detect any changes in the long dimension of the crystals during maturation, it was clearly indicated that considerable increases in dimensions perpendicular to the c-axis had occurred. A striking electron microscopic finding in sections containing crosscut crystals was the comparatively large spaces, which appeared to be empty, between the individual crystals. Since it has been proposed by others that a portion of the sparse organic matrix of the enamel is actually located within the crystals, special attention was directed to the study of that problem. At high magnification many of the crystals were found to possess a definite substructure in the form of parallel lines with a periodicity of 8.2A. These patterns were, however, interpreted as diffraction images of the 100 lattice planes and not organic structures. On the contrary, the high degree of regularity of the striations across the entire face of each crystal excludes the possibility of structural imperfections and thereby the presence of organic fibrils within the crystals. On decalcification of sections containing crosscut crystals, on the other hand, thin membraneous structures were found, which apparently had been in such intimate contact with the outer crystal surfaces that they could not be resolved in the non-decalcified specimens.

Significance to Dental Research:

It is becoming more and more evident that precise definition of the crystalline and organic components in enamel and the manner in which they are structurally mixed will be necessary for a clear understanding of how tooth substance is disintegrated in caries.

Proposed Course of Project:

It is planned to extend the study to human and/or monkey enamel with special emphasis given to the size and contents of the intercrystalline spaces before, during and after eruption.

Part B. included: Yes

Serial No. NIDR-19
1. Histology & Pathology
2. Bethesda, Md.

PHS-NIH
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Calendar Year 1962

Part B: Honors, Awards, and Publications.

Publications other than abstracts from this project.

Nylen, M. U. and Omnell, K.-Å. The Relationship Between the Apatite Crystals and the Organic Matrix of Rat Enamel, Proc. 5th International Congress for Electron Microscopy, S.S. Breese-editor, Academic Press, N.Y., QQ-4, 1962.

Scott, D. B. and Nylen, M. U. Organic-Inorganic Interrelationships in Enamel and Dentin -- a Possible Key to the Mechanism of Caries. In press.

Nylen, M. U., Eanes, E. D., and Omnell, K.-Å. Crystal Growth in Rat Enamel. In press.

Honors and Awards relating to this project.

None

PHS-NIH
Individual Project Report
Calendar Year 1962

Part A.

Project Title: Studies of the Cytodifferentiation of Odontoblasts and Ameloblasts.

Principal Investigator: Dr. H. Moe

Other Investigators: None

Cooperating Units: Department of Anatomy and Histology
Royal Dental College
Copenhagen, Denmark

Man Years (calendar year 1962):
Total: 7/8
Professional: 1/2
Other: 3/8

Project Description:

Objectives:

1. An examination of the suitability by investigations of dental tissues of different recently developed methods for fixation and embedding for electron microscopy.

2. Studies of the formation and development of endoplasmic reticulum, Golgi-elements, mitochondria and other cytoplasmic components in the ameloblasts and odontoblasts during their differentiation.

Methods Employed:

Incisors from five day old rats were used. Investigations of cytodifferentiation make heavy demands on preparation technique because cells in all developmental stages, from the undifferentiated cells at the tip of the growing end of the tooth to the fully differentiated dentin- and enamel-producing cells far removed from this area, have to be examined in one and the same tooth. In order to obtain as homogeneous a preservation as possible of the whole formative organ several fixation methods have been tried experimentally in combination with embedding in both Vestopal W and

Epon 812. Alternate series of ultrathin and 1-2 u thick sections were cut on an LKB ultrotome. A technique for staining for light microscopy of the thick sections has been developed which to a certain extent permits histochemical characterization of some of the components observed in the electron microscope.

Major Findings:

It was found that Palade's fixative and embedding in Epon yield a good tissue preservation. Good results were also obtained when similar fixation was combined with embedding in Vestopal, provided the tissue pieces were not too large. Vestopal embedded material is especially suitable for staining of thick sections for light microscopy. Material fixed following Millonig's modification of Palade's fixative has not yet been examined.

The results obtained during these early phases of the study do not allow conclusions to be drawn concerning the origin and development of the different cytoplasmic components in the odontoblasts and ameloblasts.

Significance to Dental Research:

The technical information provided from the present investigation is of very great importance to the general line of cytological work being carried on in this Laboratory. As new methods for fixation and new embedding media are proposed it is essential that they should be evaluated in the context of their usefulness in oral histology. Applications of the results are already being made in other current studies. So far as the collection of embryological data is concerned, this is obviously a longer range project than could have been completed in the available time. The ultimate significance of these findings will lie in a better understanding of the role of cells in organic matrix elaboration and calcification, as well as in the provision of information on cytodifferentiation of general biological interest.

Proposed Course of Project:

Similar studies of new techniques will be initiated whenever necessary. Dr. Moe has returned to Denmark and is continuing his work on the cytodifferentiation of ameloblasts and odontoblasts.

Part B. included: No

Serial No. NIDR-21
1. Histology & Pathology
2. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1962

Part A.

Project Title: Ultrastructural Studies of Dental Cementum.

Principal Investigator: Dr. Knut A. Selvig

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1962):
Total: 5/8
Professional: 1/4
Other: 3/8

Project Description:

Objectives:

It has been an objective of this project, which was initiated in 1961, to extend our knowledge of the mechanisms involved in the formation of cementum on human and animal teeth through studies at the ultrastructural level. A second objective has been to compare the structure and degree of mineralization of mature human cementum from teeth involved in periodontal disease with that from teeth with healthy periodontal structures.

Methods Employed:

1. Growing incisors and molars with surrounding periodontal tissues have been obtained from 8 to 78 day old mice, and processed for optical and electron microscopic examination.

2. Microradiographs have been prepared from semi-serial ground sections of human teeth of various age groups with normal periodontal structures, and from teeth involved in periodontal disease. Based upon the microradiographs it has been possible to dissect out from the ground sections small pieces of cementum with varying mineral content and structure. These specimens have been processed for electron microscopy.

Major Findings:

The interrelationship between Hertwig's epithelial sheath and early dentin and cementum formation was studied in sections of the developing end of incisors from 8-day old mice. It was found that dentin formation preceded the deposition of cementum. The cementum was formed in close contact with the dentin. The matrix of both cementum and dentin appeared to consist of collagenous fibrils invested with an amorphous ground substance. The cementum increased in thickness by deposition of crystals in and around the collagen fibrils simultaneously with a deposition of ground substance.

The cementum of mouse molars contained more densely packed collagen fibrils than that of the incisors. The Sharpey's fibers appeared to consist of bundles of parallel fibrils. As the cementum increased in thickness, crystal deposition occurred in the Sharpey's fibers as well as in the rest of the cementum matrix. Also in human teeth the Sharpey's fibers in general were found to be calcified.

By inspection of the electron micrographs and by electron diffraction, it was determined that the hydroxyapatite crystals of the cementum in general were arranged with their c-axes parallel to the collagen fibrils. This arrangement is similar to that found in bone and dentin. Microradiographs of ground sections from human teeth have revealed a zone of increased mineral content on the surface of the cervical cementum in many teeth extracted because of periodontal disease. This confirms the results from earlier chemical analysis of such teeth by the investigator.

Significance to Dental Research:

This project has yielded basic knowledge about the normal formation and mineralization of cementum. A baseline has thus been established for a study of alterations in structure and mineralization of this tissue connected with periodontal disease.

Proposed Course of Project:

This project will be continued at the University of Bergen, Bergen, Norway.

Part B. included: Yes

PHS-NIH
Individual Project Report
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Part B: Honors, Awards, and Publications.

Publications other than abstracts from this project.

Selvig, K. A. Chemical Analysis and Microradiography of Cementum and Dentin from Periodontally Diseased Human Teeth, in press.

Selvig, K. A. Electron Microscopy of Hertwig's Epithelial Sheath and of Early Dentin and Cementum Formation with Mouse Incisor, in press.

Honors and Awards relating to this project.

None

PHS-NIH
Individual Project Reports
Calendar Year 1962

Part A.

Project Title: Studies Concerning the Ultrastructure of Bacteroides: A Genus of Bacteria Found in the Oral Cavity.

Principal Investigator: Dr. H. A. Bladen

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1962):

Total:	1 5/8
Professional:	1/2
Other:	1 1/8

Project Description:

Objectives:

To determine the ultrastructure of Bacteroides, as well as intercellular relationships.

Methods Employed:

Bacterial cultures were grown in a synthetic medium under anaerobic conditions. After various incubation periods, cultures were fixed, dehydrated and embedded in different embedding media for electron microscopy. Thin sections were cut on an LKB ultratome, stained with uranyl acetate or phosphotungstic acid and examined electron microscopically.

Major Findings:

1. General Morphological Structure.

A number of different Bacteroides strains have been examined to date. A definite cell wall-space-cytoplasmic membrane arrangement can be observed. The cell wall is composed of three structures, two dense lines (40A) separated by a less dense area of approximately the same size.

The cell wall is separated from the cytoplasmic membrane by a distance of approximately 250 A. Within this spatial arrangement appear to be small bridges connecting the cell wall to the cytoplasmic membrane. There is some question as to whether these structures are actually bridges (connecting tubes) or are artifacts due to parting of a normally closely apposed cell wall from the cytoplasmic membrane, the bridges being areas where adhesion was greatest between the two structures.

The cytoplasmic membrane is innermost of the observable membranes. It appears as a three unit structure, two dense lines separated by a less dense area, very similar to the cell wall. However, the outer dense line appears unusually thick and high resolution micrographs suggest that this structure may be composed of two dense lines separated by a very narrow less dense area. Thus, the cytoplasmic membrane may actually be a five unit structure composed of three dense lines separated by two less dense areas.

2. Demonstration of an unusual ultrastructure found in Bacteroides: A conjugatory bridge?

During electron microscopic study of the ultrastructure of a strain of Bacteroides, certain morphological entities which might be interpreted to represent conjugatory bridges were observed in thin sections. Cells were united, not by simple fusion of the cell walls, but by tubes or bridges which varied from 125 to 500 Å in diameter and from 1500 to 9000 Å in length. Occasionally, a single dense line 40 A wide and continuous with the outer layer of the cell wall formed the boundaries of the bridge. More frequently, the bridge appeared to be bordered by two double structures, each approximately 110 Å wide and resembling the usual dense-light-dense arrangement of the cell wall with similar dimensions. It has not yet been possible to determine whether these double structures are continuous with the similar cell wall structures; however enough similarity exists to permit this as a tentative conclusion.

Some bridges appeared to be coiled between cells. This spiral configuration probably resulted from twisting during preparatory procedures and since cellular connections are still evident in some cases, these unusual structures would serve to further confirm the conclusion that the boundaries of the bridges are composed of the cell walls of the organisms.

Conjugation has been known to occur in bacteria for approximately a decade, but the extreme rarity of this event has prohibited observation in almost all cases. If these electron

micrographs actually demonstrate various stages of conjugation, it is the first time such visual evidence has been reported.

Significance to Dental Research:

Studies concerning the basic morphology of Bacteroides, a genus of bacteria normally found in the oral cavity, may lead to further understanding of fundamental problems in relation to the oral microbiota. The study concerning a conjugatory bridge has broad genetic implications.

Proposed Course of Project:

An effort will be made to resolve the problems concerning the existence of bridges between the cell wall and cytoplasmic membrane as well as the five unit cytoplasmic membrane. Further studies are to be initiated concerning cellular morphology of related groups of microorganisms. Investigation of the intercellular bridges will be continued.

Part B. included: Yes

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B: Honors, Awards, and Publications.

Publications other than abstracts from this project.

Bladen, H. A. Demonstration of an Unusual Ultrastructure Found in Bacteroides: A Conjugatory Bridge? J. Bact. (in press).

Honors and Awards relating to this project.

None

Serial No. NIDR-23
1. Histology & Pathology
2. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1962

Part A.

Project Title: Secondary Collaborative Projects and Training Activities.

Principal Investigators: Dr. M. U. Nylen
Dr. D. B. Scott

Other Investigators: Dr. H. A. Bladen
Dr. D. V. Provenza
Dr. A. Qureshi

Cooperating Units: See below

Man Years (calendar year 1962):
Total: 3 3/4
Professional: 2
Other: 1 3/4

Project Description:

Objectives, Methods Employed and Major Findings:

Each year a relatively large number of requests is received for applications of biophysical methods to problems under investigation in other laboratories. It has long been accepted that such operations as electron microscopy cannot be adapted to routine service function, primarily because each type of specimen to be studied presents new and usually difficult problems in handling, and because the microscopic observations must be made by an experienced professional investigator. Inasmuch as many of the requests represent worthwhile problems, and since most relate in some fashion to the general program of this Laboratory, the staff has undertaken to offer as much secondary collaboration as seems feasible. The magnitude and success of these projects, listed below, have reached a level at which it is now necessary to consider this activity as a regular part of the Laboratory program.

Collaborative Studies

1. Studies of calculogenesis: primarily electron microscopy and diffraction of intra-oral deposits formed *in vivo* on polyester strips attached to teeth. With Dr. H. A. Zander, Eastman Dental Dispensary, Rochester, New York. To be continued; manuscript on results to date, completed for submission.

2. Study of epithelial tissue characteristics, intra- and extracellular of the gingiva attached to enamel and cementum in human and monkey material. With Dr. H. A. Zander, Eastman Dental Dispensary, Rochester, New York. Techniques developed, preliminary electron microscopic survey made; to be continued.

3. Mineralization of microorganisms in intraperitoneal implants, studied by electron microscopy of sectioned material and direct X-ray diffraction of recovered implants. With Dr. A. A. Rizzo, Laboratory of Microbiology, NIDR. Manuscript on work to date in preparation; work to be continued.

4. Mineralization of rat aorta *in vitro*. Exploration of initial nucleation sites and calcification sequence by electron microscopy and microradiography. With Dr. G. Martin, Laboratory of Biochemistry, NIDR. Manuscript in press; work to be continued, as well as extended to human material.

5. Study of calcareous corpuscles in tapeworms; electron microscopy, electron diffraction and microradiography of isolated corpuscles and sectioned specimens. Manuscript in press deals with a large-scale study of several hundred samples treated chemically and thermally to permit postulation of the existence of inorganic complexes in the amorphous mineral present in corpuscles in the natural state. With Dr. T. von Brand, Laboratory of Tropical Diseases, NIAID. To be continued.

6. Morphology of the flame cells in parasitic worms. This study is a by-product of the preceding one, and has received special attention because of its interest in parasitology. These cells have a most interesting and complex structure, part of which is cilia-like, and electron microscopic examination is very demanding technically. Aside from their biological interest, these cells have proved most useful as test objects in the

evaluation of new fixation and embedding techniques in progress in this Laboratory. With Dr. T. von Brand, Laboratory of Tropical Diseases, NIAID. Study almost completed.

7. Study of demineralization at neutral pH in molluscs by glandular action of the accessory boring organ in the bivalve. Electron microscopy, optical microscopy and electron diffraction of replicas and pseudo-replicas of affected shells in in vitro experiments; microradiography of actual bored holes in shells; electron microscopy of sectioned boring organs in active and inactive secretory states. With Dr. M. R. Carriker, Marine Biological Laboratory, Woods Hole, Massachusetts. Manuscript in preparation reporting etching patterns on shells, decalcification sequences, non-specificity to calcium carbonate (also attacks calcium phosphate of bone and tooth in vitro), and nonreactivity with organic matrix. Work to be continued largely concerns ultrastructure of secretory cells, which are of interest not only as related to decalcification mechanisms, but also in comparison to other cells such as those involved in calcification.

8. Studies concerning the presence and ultrastructure of the lactic dehydrogenase agent, presumably a virus associated with animal tumors. Electron microscopic observation of micro-drop suspensions or sectioned pellets of ultracentrifugally purified particle-containing material isolated from mouse plasma; shadowed, or negative or positive stained preparations. With Dr. A. Notkins, Laboratory of Microbiology, NIDR. Particles 25-40 m u have been observed and are being characterized structurally. To be continued.

9. Studies of pneumocystis carennii. This study was generated from prior experiences and associations with Dr. C. Zierdt, Clinical Pathology Department, Clinical Center. The latter has grown, subcultured and concentrated for the first time what appears to be the causative organism in the pheumocystitis which can be induced in rats by cortisone injection, and which resembles the organism seen in human specimens. Optically this "organism" is granular and quite large (15-20 u in diameter). The present electron microscopic study is being conducted to define clearly the structure of the organism in an attempt to determine its proper phylogenetic classification, which has not hitherto been possible. To be continued.

Training Activities

In this area staff members are involved in the training of three professionals in biophysical methodology, principally electron microscopy.

1. Dr. H. A. Bladen, a microbiologist who joined our permanent staff, has been in training for one year, and has made excellent progress. As a professional, he has been able to assume responsibility for an independent project on the ultrastructure of Bacteroides, and in collaboration with other investigators in this Laboratory and others he has participated productively in numerous of the projects listed in the present report (3,4,8,9).

2. Dr. D. V. Provenza, Special Fellow, Professor and Head of the Department of Histology at the University of Maryland Dental School, is on sabbatical leave for a training period of one year in electron microscopy. Inasmuch as he is an experienced histologist, he is making rapid progress technically and has been able to assume responsibility for work on the cytological studies on the secretory cells mentioned as project 7 in this report. He has independently done the required preliminary optical microscopy and histochemistry, and with staff assistance is employing the same material as specimens for his training in fixation, embedding, sectioning and electron microscopic techniques. In addition, he is learning associated methods used in other studies in the laboratory.

3. Dr. A. Qureshi, an M.D. with a graduate degree in gross anatomy, is being trained in electron microscopy as a Visiting Fellow in the NIH Visiting Program. In this case the training program has been adapted to provide both experience in technique and an introduction to ultrastructural cytology. At this time, Dr. Qureshi has reached the point where it should be possible for her to begin participating productively in a research project.

Along lines of short term requests for assistance in technique, approximately a dozen individuals from various institutions have spent periods from two days to a week learning particulars of essentially all electron microscopic, microradiographic and darkroom procedures.

Three COSTEP students served in trainee capacity, doing junior professional work.

Significance to Dental Research:

The importance of making collaborative effort available is self-evident, as is the benefit derived by those receiving training. In addition, it is felt that opportunities are provided to the staff of our Laboratory, professional and technical, both for broadening their experience in the general field of biophysical instrumentation and for contributing useful information related in most part to our interests in the oral tissues, especially with respect to the mineralization phenomena.

Proposed Course of Project:

As indicated above, collaborative work will continue on numerous problems. Training activities will also be continued and at mid-year an NIH Research Associate will join the program.

Part B. included: Yes

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Part B: Honors, Awards, and Publications.

Publications other than abstracts from this project.

Rizzo, A. A., Martin, G. R., Scott, D. B. and Mergenhagen, S. E. Mineralization of Bacteria. Science 135:439-441, 1962

Scott, D. B. Features of Extracellular Matrices Which Mineralize. Proc. V. Int. Congress for Electron Microscopy, Academic Press, New York, Section QQ3, 1962.

Scott, D. B., Nylen, M. U., von Brand, T. and Pugh, M. H. The Mineralogical Composition of the Calcareous Corpuscles of Taenia Taeniaeformis. (in press) J. Exp. Parasit.

Theilade, E., Theilade, J., and Scott, D. B. The Morphology of an Anerobic Oral Filamentous Microorganism as Revealed by Electron Microscopy, (in press).

Bladen, H. A., and Martin, G. R. Preferential Mineralization of Elastin in a Matrix also Containing Collagen. Proc. V. Int. Congress for Electron Microscopy, Academic Press, New York, 1962, Vol. 2.

Martin, G. R., Schiffman, E., Bladen, H. A., and Nylen, M. J., Chemical and Morphological Studies on the In Vitro Calcification of Aorta, J. Cell Biol. (in press).

Honors and Awards relating to this project.

None

Serial No. NIDR-24
1. Histology & Pathology
2. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1962

Part A.

Project Title: Crystal Chemistry of Calcified Tissue and Related Compounds.

Principal Investigator: Dr. A. S. Posner

Other Investigators: Dr. E. D. Eanes
Dr. I. Zipkin
Dr. R. C. Likins
Dr. J. Menczel

Cooperating Units: None

Man Years (calendar year 1962):

Total:	3 1/4
Professional:	1 3/4
Other:	1 1/2

Project Description:

Objectives:

Elucidation of the crystal chemistry of the mineral and protein portions of bone and teeth. This project includes work on (a) the crystal structure (i.e. atomic and/or molecular arrangement) and (b) the crystal texture (i.e. size, shape, orientation, etc.) of these materials and the effect of these factors on the properties and behavior of bone and tooth substance.

Methods Employed:

The principal method used in this project is X-ray diffraction. Single crystal X-ray techniques are employed to investigate isomorphous substitution in mineral and synthetic apatites. Powder X-ray diffraction techniques, using both film and counter diffractometers, are employed for phase studies, crystal size and shape analysis, and crystal perfection studies. Microscopic methods are used as adjuncts.

Major Findings:

1. It was shown by X-ray diffraction that fluoride ingestion in humans and laboratory rats tended to improve the crystallinity of the bone apatite crystals. The crystals of bone apatite tended to be larger and more perfect with increased fluoride content. Detailed X-ray studies show that the hexagonal bone apatite crystals did not change in size or perfection in the c-axis direction but grew in the direction perpendicular to this axis. Preliminary X-ray low angle scattering results indicate that fluoride is causing growth in the small (i.e. a-axis) direction in the crystal. This effect of fluoride on bone crystallinity may be explained on the basis of an increase in nucleation and growth rates due to the presence of fluoride in the serum.

2. Structure studies have shown the chloride ion substitutes in hydroxyapatite in a different position from the fluoride or hydroxyl ion. The hydroxyl and fluoride ion, on the other hand, occupy identical structural position when substituted for one another.

Significance to Dental Research:

1. Since bones and teeth share some common properties, these findings on fluoride may help explain why enamel of fluoride-protected teeth is more resistant to caries. Findings revealed that fluoride stabilized bone by (a) increasing crystallinity and (b) substituting for hydroxyapatite thus forming a less soluble fluoride substituted apatite.

Proposed Course of the Project:

The exact role of fluoride in synthetic apatite crystal nucleation and growth will be studied by X-ray diffraction techniques. In addition the effect of crystal texture (size, shape, etc.) on the chemical and physical properties of synthetic and biological apatites will be investigated.

Part B. included: Yes

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Part B: Honors, Awards, and Publications.

Publications other than abstracts from this project.

Zipkin, I., Posner, A. S. and Eanes, E. D. Effect of Fluoride on the X-ray Diffraction Pattern of the Apatite of Human Bone. Biochim. Biophys. Acta 59:253, 1962.

Posner, A. S., Eanes, E. D., Harper, R. A. and Zipkin, I. X-ray Diffraction Analysis of the Effect of Fluoride on Human Bone Apatite. Arch. Oral Biol. (in press).

Posner, A. S. The Structure of the Mineral Portion of Teeth. Adhesive Restorative Dental Materials. Proceedings of a Workshop, compiled and edited by R. W. Philips and G. Ryge, Owen Litho. Service, Spencer, Indiana, 1962.

Menczel, J., Posner, A. S., Pakis, G. and Likins, R. C. Comparative Fixation of Sr^{89} and Ca^{45} by Calcified Tissues as Related to Fluoride Ingestion. Proc. Soc. Exp. Biol. and Med. 110:609, 1962.

Menczel, J., Schraer, R., Pakis, G., Posner, A. S. and Likins, R. C. Effects of Dietary Calcium and Fluoride on Skeletal Uptake of Ca^{45} in Rats. Proc. Soc. Exp. Biol. and Med., in press.

Posner, A. S. and Eanes, E. D. Fluoride in Bone and Teeth. Norelco Reporter, (in press).

Honors and Awards relating to this project:

Claude Bernard Guest Professorship at the Institute of Experimental Medicine and Surgery, University of Montreal, November, 1962.

Serial No. NIDR-25
1. Histology & Pathology
2. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1962

Part A.

Project Title: Infrared Absorption Spectrophotometric Studies on Calcified Tissues and Related Synthetic and Natural Compounds.

Principal Investigator: Dr. Aaron S. Posner

Other Investigators: None

Cooperating Units: Professor E. R. Lippincott, Department of Chemistry, University of Maryland; Dr. W. H. Emerson, School of Dental Medicine, Tufts University.

Man Years (calendar year 1962):

Total:	3/4
Professional:	1/4
Other:	1/2

Project Description:

Objectives:

To ascertain the chemical structure of the mineral portion of teeth and bones and to study the chemical and physical relationship between the mineral and protein phases of these calcified tissues. The study includes the properties of normal and pathological tissue as well as the effect of certain factors such as diet, age, and disease on the structure and properties of these tissues.

Methods Employed:

Major emphasis is placed on infrared spectrophotometry. This work will be correlated with other physical chemical techniques.

Major Findings:

1. A method was developed to study samples of synthetic and biological apatites by infrared methods using the normal potassium bromide disc mounts. Until recently it was not possible to use potassium bromide mounts in infrared studies

because of the interference of water absorption in the region of the hydrogen bond absorption. New drying techniques were developed which would allow the use of this simple technique.

2. Preliminary results on autopsied fluoride-containing bones indicate that there is a perfection of the ordinarily calcium deficient atomic structure of bone apatite with an increase in fluoride content.

3. Results on dental enamel (human and rat) showed that the apatite portion of this hard tissue is calcium deficient and contains hydrogen bonds between the orthophosphate oxygens. There was a range in stoichiometry, described by the formula $Ca_{10-x}H_{2x}(PO_4)_6(OH)_2$, from $2x = 0.60$ to $2x = 0.13$, where x is the average number of Ca atoms missing per unit cell and 2x the number of hydrogen atoms included as hydrogen bonds for electrical neutrality.

Significance to Dental Research:

These results show that the major constituent of enamel is some form of calcium deficient apatite. Pure apatite (no calcium ions missing) is more chemically inert than calcium deficient apatite and so it seems clear that it may be possible to stabilize enamel by chemical treatment directed toward producing a biological apatite with minimal calcium deficiencies.

Proposed Course of the Project:

The presence of hydrogen bonds in enamel will be used to chart the change in stoichiometry of enamel apatite with age, disease and dietary changes. Work will be continued on synthetic calcium phosphates to delineate the exact nature of hydrogen bonding in all of these substances.

Part B. included: Yes

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Part B: Honors, Awards, and Publications.

Publications other than abstracts from this project.

Stutman, J. M., Posner, A. S. and Lippincott, E. R. Hydrogen Bonding in the Calcium Phosphates. Nature 193, 367, 1962.

Honors and Awards relating to this project.

None

Serial No. NIDR-26
1. Histology & Pathology
2. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1962

Part A.

Project Title: Histochemical Studies of Connective Tissues

Principal Investigator: Dr. Harold M. Fullmer

Other Investigators: Dr. George Martin

Cooperating Units: None

Man Years (calendar year 1962):
Total: 3
Professional: 1
Other: 2

Project Description:

Objectives:

To determine the chemical composition and reactive groups in normal and diseased connective tissues through the use of histochemical methods.

Methods Employed:

Human and animal tissues are obtained from autopsy and surgical material. Specimens are then prepared for histochemical analysis and optical microscopy. Sections may be cut (a) in a cryostat, (b) frozen after fixation in various fixatives, (c) after freeze-drying, or (d) after routine chemical fixation, dehydration and paraffin embedding. Under controlled conditions, the sections are exposed to various reagents designed to determine the side and quantity of an enzyme, reactive group or specific substance.

Major Findings:

In 1958, two new stains, i.e. the peracetic acid-aldehyde fuchsin-Halmi and the peracetic acid-orcein-Halmi, were developed which led to the discovery of a new connective tissue fiber, which has been called oxytalan. It has been found in periodontal membranes, ligaments and mucous connective tissues. Thus, certain specialized

connective tissues have oxytalan fibers as well as previously recognized fibers. New reparative tissue that develops subsequent to periodontal disease, dental granulomas and radicular cysts contain oxytalan fibers. Periodontal membranes that thicken as the result of sclerodermatous involvement contain proportionately increased numbers of oxytalan as well as collagen fibers. It has also been found that the connective tissue stroma of ameloblastomas contain oxytalan fibers. These data serve to indicate that the production of oxytalan fibers in certain anatomical locations is genetically determined.

Comparative anatomical, embryological and histochemical studies of the periodontia of dogs, hog, sheep, cow, deer, rabbit, guinea pig, mouse, rat, monkey, and man indicate that oxytalan fibers belong to the elastic family of connective tissue fibers. The observation that some elastic fibers appear to have developed from oxytalan fibers in periodontal membranes involved with scleroderma in man reinforce this view.

The peracetic acid-aldehyde fuchsin-Halmi stain is the first stain to demonstrate pre-elastic fibers.

A histochemical study of periodontal disease in human autopsy material has been progressing for several years. Most pathologists have previously tended to favor the view that hard and soft connective tissues are lost in periodontal disease as a direct result of an inflammatory process. From the present work attention has been called to the fact that frequently soft connective tissues and bone undergo degradation at a site distant from the inflammatory focus without concomitant inflammation. This results in a weakened periodontal membrane in advance of the inflammatory process which is thereby more easily destroyed.

Although it is well known that bone is continuously being produced and resorbed, and that collagen probably has a low turnover rate as compared to other body proteins, practically nothing is known about the factors that foster the loss of either collagen or bone at any particular site. A recent report, which we have confirmed, indicates the preservation of many enzymes in properly prepared tissues decalcified with cold neutral ethylenediaminetetraacetic acid solutions. Preserved enzymes include those giving evidence of activity of the citric acid cycle, pentose cycle, glycolysis and fatty acid oxidation. Our studies indicate that the application of histochemical methods to this problem will allow one to chart metabolic behaviour of individual cells and to correlate it with the histological and pathological situations. In collaboration with Dr. R. D. Lillie, the most selective stain for nucleo-protein in use today has been developed. This stain will be particularly

useful for all those cytologists, geneticists, pathologists and others working with chromosomes, DNA synthesis, and cancer. Collaborative work with Dr. George Martin has been progressing for several years. Several experiments have been conducted to determine the mechanism of action of Vitamin C. Scorbutic guinea pigs have been given various drugs to determine anti-scorbutic activity. D-ascorbic acid, 5-methylcholanthrene and 5-benzpyrene have exhibited antiscorbutic activity. It has not been possible to advance a hypothesis to explain the antiscorbutic activity of the latter two.

Significance to Dental Research:

Periodontal disease ia a major cause for the loss of teeth. At histochemical examination of this disease has direct application to dentistry. Work on oxytalan fibers has considerably broadened our view of elastic tissues. It is now apparent that a spectrum of elastic tissues exists. Vitamin C has been prescribed to individuals with gingival disorders for many years. Elucidation of the mechanism of action of Vitamin C would provide a rational approach to this therapy as well as to the treatment of other deficiency defects.

Proposed Course of Project:

Continuation along the same lines. In addition, collaborative exploratory work will be directed toward identifying the cytological localization of several dehydrogenases at the electron microscopical level.

Part B. included: Yes

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1. Histology & Pathology
2. Bethesda, Md.

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Part B: Honors, Awards and Publications

Publications other than abstracts from this project.

Fullmer, H. M. A Critique of Normal Connective Tissues of the Periodontium and Some Alterations with Periodontal Disease, J. Dent. Res. 41:223-229, 1962.

Fisher, A. K. and Fullmer, H. M. Oxytalan Fibers in Ameloblastomas. Oral Surg., Oral Path., Oral Med. 15:246-248, 1962.

Fullmer, H. M. and Witte, W. Periodontal Membrane Affected by Scleroderma: A Histochemical Study. Arch. Path. 73:184-189, 1962.

Wertheimer, F. W. and Fullmer, H. M. Morphological and Histochemical Observations on the Human Dental Cuticle. J. Periodontol. 33:29-39, 1962.

Fullmer, H. M. and Lillie, R. D. Dilute Unmordanted Hematoxylin as a Stain for Basic Nuclear Protein. J. Histochem. Cytochem. 10:502-503, 1962.

Wertheimer, F. W. and Fullmer, H. M. Histochemical Observations of Cuticular Bodies in Radicular Cysts. J. Periodontol. 33: 1962, in press.

Fullmer, H. M. The Use of Histochemistry in Oral Pathology. Textbook of Oral Histology, edited by V. Provenza, Lea & Febiger, in press.

Fullmer, H. M. The Use of Histochemistry in Oral Histology. Textbook of Oral Pathology, edited by Tiecke, R. McGraw Hill, in press.

Fullmer, H. M. The Histochemistry of Connective Tissues. International Review of Connective Tissue Research, edited by D. A. Hall, Acad. Press. in press.

Honors and Awards relating to this project.

Fulbright Research Scholar at the University of Adelaide, Adelaide, Australia for the year 1962.

Serial No. NIDR-27
1. Histology & Pathology
2. Bethesda, Md.

PHS-NIH
Individual Project Report
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Part A.

Project Title: Experimental Dental Caries

Principal Investigator: Dr. P. H. Keyes

Other Investigators: Dr. R. J. Fitzgerald
Dr. H. V. Jordan

Cooperating Units: National Childrens' Cardiac Hospital, Miami, Florida.

Man Years (calendar year 1962):

Total:	2 1/2
Professional:	3/4
Other:	1 3/4

Project Description:

Objectives:

The studies undertaken on this project extend prior work on experimental caries and were directed mainly to the disclosure of conditions affecting the induction and control of the disease in albino hamsters.

Methods Employed:

1. The cariogenic potential of several new bacterial isolates was tested. These included a new streptomycin-resistant mutant, strain 3720, a streptococcus isolated from a golden hamster which developed spontaneous disease, streptococci isolated from the mouth, heart, and lungs of a hamster which had been sent to colleagues at the National Childrens' Cardiac Hospital in Miami, Florida, and other animal and human strains of streptococci which fluoresce after treatment with the "specific"antibody.

2. Several agents: bacitracin, cetyl pyridinium chloride, sodium fluoride, various carbonates, ash, etc., were tested as inhibitors and agents to reduce the cariogenic microflora.

3. The role of liver fractions in the caries-test diet was studied and led to a new formula for an experimental ration.

4. Material accumulated several years ago in studies of caries susceptibility in various strains of rats was evaluated in collaboration with a COSTEP student.

Major Findings:

1. The streptomycin-resistant streptococcus isolated by R. J. Fitzgerald (Gnotobiotic Section) and designated as strain 3720 was highly pathogenic and established itself readily in animals known to carry other cariogenic strains. It has proved useful as a tracer organism in several studies. Some strains of streptococci isolated by H. V. Jordan and R. J. Fitzgerald have not only shown the capacity to induce caries but also a possible potential for gingival ulceration.

   Several streptococcal strains studied by the National Childrens' Cardiac Hospital have fluoresced when treated with an antibody produced by pure cultures of our known cariogenic streptococci. However, when the cariogenic potential of these strains has been tested in our laboratories at NIDR no highly active disease has followed their inoculation. One human strain may have a cariogenic potential but further studies will be necessary to determine this for certain.

2. The agents tested for caries inhibition in albino hamsters produced variable results: It was not possible to test adequately bacitracin because the animals developed a fatal diarrhea in each instance. Previous findings in rats, however, suggest a caries inhibitory potential sufficient to justify clinical trial. (Fitzgerald, 1955, "Influence of Antibiotics on experimental Rat Caries"). Cetyl pyridinium chloride was not beneficial as applied in the drinking water, although it suppressed streptococcal growth in vitro (H. V. Jordan). In general the carbonates and ashes were associated with less caries activity.

3. The 4% liver powder fraction formerly used in the experimental diet was found to be "toxic," and yeast powder was found to be highly beneficial. The new diet contains 3% yeast and 1% dried liver substance. A marked improvement in animal growth and well being has followed this change. Caries activity has been greatly enhanced following the use of this new diet.

4. The study of caries in rat jaws from several strains indicates that while animals have inherent differences which affect the induction and course of the disease, the nature of experimental challenge (environmental, dietary, and microbiological)

determines the outcome of the results. Strains of rats previously characterized as "resistant" have developed caries when tested under other conditions. Morphological differences in the teeth account for less activity in some instances, but such factors probably should not be characterized as resistance.

Significance to Dental Research:

The pathogenic potential of streptococci and their apparent specificity in caries etiology needs further investigation. Why are these organisms causing caries, and how do they do so? What does the environment of the tooth and its chemical properties mean to the organisms? Efforts to answer these questions should receive high priority in support! The new isolate of mutant streptococci have overcome previous difficulties and offer investigators a new tool for studying various ecological factors related to caries.

It has become apparent that experimental animals are not suitable for the study of certain drugs and methods of therapy in caries control. Drugs which cause secondary disorders, i.e. bacitracin, cannot be properly tested, and those which are not stable in the entire elimentary canal cannot be adequately assessed because of the copraphagous habits of the animals which results in continuous reinfection of the mouth from feces. These findings are significant because they show some of the limitations to the use of experimental animals in studies of caries inhibitors. But in the clinical situation brief topical applications of drugs will probably not be too beneficial because sufficient concentration of drug will not be present for a sufficient period of time. Thus, certain chemotherapeutic agents can only be tested under other conditions or in human subjects. Improved methods for characterizing the human oral microbiota are urgently needed so that various drugs and methods of therapy can be assessed. By modifications of present day in vitro methods, it may be possible to obtain useful information, at least a preliminary type.

The place small fractions of the diet play in animal well being and the course of caries was well illustrated by the disclosure that the liver powders as used had an adverse effect on animal health. The caries was less active than usual, probably because of reduced food intake. This observation is important in that it relates to the interpretation of animal studies.

The findings in rats should help to clarify several points debated for the last three or four years. We need better agreement on the meaning of the terms resistance, caries inactivity, susceptibility, etc.

Proposed Course of Project:

To continue with studies which enhance an understanding of the role various factors contribute in caries activity. To initiate a program of human clinical study which will test the feasibility of controlling caries by the use of a chewing gum containing the antibiotic, bacitracin, and the salt, sodium fluoride.

Part B. included: Yes

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1. Histology & Pathology
2. Bethesda, Md.

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Part B: Honors, Awards, and Publications.

Publications other than abstracts from this project.

Keyes, P. H. and Fitzgerald, R. J. Dental Caries in the Syrian Hamster. IX., Archives Oral Biology, 7:267-278, 1962.

Keyes, P. H., Fitzgerald, R. J., Jordan, H. V. and White, C. L. The Effect of Various Drugs on Caries and Periodontal Diseases in Albino Hamsters. Archives Oral Biology. Special supplement, 7:123-141, 1962.

Honors and Awards relating to this project.

Joachim International Prize. Co-winner, Dr. R. J. Fitzgerald, awarded at the International Dental Federation, Koln, Germany, July, 1962.

First prize for Scientific Exhibits presented by Federal Agencies at the Annual Meeting of the American Dental Association, Miami, Florida, November, 1962.

Serial No. NIDR-28
1. Histology & Pathology
2. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1962

Part A.

Project Title: Experimental Periodontal Disease.

Principal Investigator: Dr. P. H. Keyes

Other Investigators: Dr. H. Jordan
Dr. A. Howell
Dr. R. J. Fitzgerald

Cooperating Units: None

Man Years (calendar year 1962):
Total: 1
Professional: 1/4
Other: 3/4

Project Description:

Objectives:

To study the induction of an experimental type of periodontal pathosis in hamsters and to examine the gross pathological, histological, and bacteriological characteristics.

Methods Employed:

Hamsters are maintained on the diet used for caries studies. Periodically they are examined alive to determine if they are developing periodontal disease either from "natural" modes of infection or as the result of inoculations of pure cultures of specific bacteria. The cultures used have been largely prepared by H. V. Jordan, Gnotobiotic section. Routine histological methods have been used to prepare jaws for microscopic study, and various culturing technics are used to study the organisms present (H. V. Jordan and A. Howell).

Major Findings:

Spontaneous periodontal disease has developed in a family of hamsters whose great grand dams were treated with penicillin in order to depress the cariogenic microbiota. This disease has been

perpetuated by breeding females with a history of this disease. Scrapings of subgingival plaque material taken from animals with this disease induce similar lesions in noninfected host animals. A number of organisms have been isolated from such plaque material (H. V. Jordan and A. Howell); however, only filamentous forms have induced typical periodontal disturbances like those found in naturally infected animals.

The pathogenic filaments have been labelled by an induced resistance to the antibiotic, terramycin. These labelled mutants induce the disease and can be recovered and identified from inoculated animals. The pathological characteristics of this disease have been studied grossly and by simple histological techniques.

Significance to Dental Research:

These findings will be reported at the March, 1963, meeting of the International Association of Dental Research and are likely to stimulate considerable interest. This observation is similar to that made a few years ago in the field of experimental dental caries, i.e. apparently this is a type of periodontal disease which has an infectious component, now identified as a unique type of filamentous organism (Howell and Jordan).

Proposed Course of Project:

Albino hamsters are available which do not harbour either caries inducing or periodontal disease inducing organisms. Work will be directed to the study of this disease as a specific microbial infection to study factors which are conducive to its induction and progression, and also those which prevent or inhibit the disease. These observations open the way for many studies related to the specific pathogenicity of various organisms and to the characterization of the various changes they induce in the tissues of the host animal. This disease will undoubtedly be studied from the viewpoint of the bacteriologist, the pathologist, the histochemist, and electron microscopist, and the nutritionist.

Part B. included: No

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BIOCHEMISTRY:

29. J. E. Folk: Protein and Amino Acid Chemistry. I. Proteolytic Enzymes: Chemical and Molecular Studies. II. Blood Clotting: Molecular Mechanisms.

30. A. H. Mehler: Enzyme Structure and Mechanism of Action.

31. G. R. Martin: The Molecular Basis of Connective Tissue Metabolism and Function.

32. M. S. Lewis: Physical Chemistry of Proteins.

33. R. C. Likins: Biochemical and Biophysical Studies of Calcification.

34. F. J. McClure: The Cariostatic Effect of Phosphate.

35. K. A. Piez: Analytical and Structural Aspects of Collagen and Other Proteins in Relation to Function.

36. S. A. Narrod: Metabolic Controls.

37. I. Zipkin: Biochemistry and Physiology of the Salivary Glands and their Secretions.

38. I. Zipkin: Factors Affecting Calcification of the Skeletal and Dental Tissues.

39. F. H. Bergmann: Studies on Soluble Ribonucleic Acid.

40. L. F. Hass: Characterization of the Structure and Function of Muscle Aldolase by Physiochemical and Enzymatic Methods.

41. F. J. Kendrick: Prenatal Influences on Fetal Development.

42. C. T. G. King: The Effect of Prenatal Factors on Fetal Development and other Conditions Influencing Oral Diseases.

43. A. Peterkofsky: I. The Enzymology of Vitamin B_{12} and Its Relation to One-Carbon Metabolism. II. Mechanism of Enzyme Action.

44. E. Schiffmann: Studies on the Mechanism by which Endotoxins Induce Non-specific Resistance in Mammals.

45. E. Schiffmann: Factors in the Calcification of Mammalian Aortas and Certain Matrices.

Annual Report of the Laboratory of Biochemistry
National Institute of Dental Research
Summary Statement

PROTEIN CHEMISTRY

The activities of the Section on Protein Chemistry have centered principally on the structure and function of connective tissue proteins.

In a recently completed study, it was demonstrated that collagens from several species (dogfish, carp, rat, chick embryo, and guinea pig) and several tissues (skin, tendon, and bone) have the same general structure, i.e., in each case the molecule contains three subunits, two of one type and one of another type. Designated $\alpha 1$ and $\alpha 2$, these subunits have different, but related, amino acid compositions. The structure is stabilized by intramolecular crosslinks between chains which form at a rate and to a degree characteristic of the tissue.

LATHYRISM

Toxic agents such as β-aminopropionitrile injected into experimental animals develop symptoms which are related to a connective tissue disease called lathyrism. Following an earlier demonstration, that collagen from lathyritic animals contains a much reduced content of crosslinked subunits, it was established by isotope and tissue culture technics that the primary effect of lathyritic agents is to break or prevent the formation of the crosslinks. The gross symptoms develop later as a result of the incomplete maturation of the collagen fibrils and the consequent loss of integrity of the tissue.

The results obtained from the study of lathyrism indicate that intramolecular (and perhaps intermolecular) crosslinking is an important aspect of connective tissue maturation. Further, the observation that the degree of crosslinking varies from one tissue to another indicates that this may have an important relation to function. Presumably, either hypo- or hypercrosslinking might produce a connective tissue disorder.

COLLAGEN;-MOLECULAR WEIGHT, STRUCTURE AND FUNCTION

The molecular structure of collagen is established, in part, by precise molecular weight data on both the native molecule and its subunits. Owing to aggregation phenomena, it has been extremely difficult to obtain accurate molecular weights of these complex compounds. Recent work has shown, however, that sedimentation equilibrium technics offer high precision and give the best estimate of homogeneity of a sample. Whereas previously the best collagen samples had been shown to have molecular weights near 360,000, the new, more accurate, value now stands at 300,000. In confirmation of our proposed subunit structure, $\alpha 1$ and $\alpha 2$ have molecular weights of about 100,000. The covalently linked dimers formed from $\alpha 1$ and $\alpha 2$ have about twice this weight, as expected.

The fact that collagen is found throughout the animal kingdom and may have diverse functions in a single species (for example, tendon, skin, bone, and dentin have somewhat different properties and functions), indicates that relatively minor variations within its basic molecular structure could affect its properties. In addition to the possible role of crosslinking already mentioned, amino acid content and amino acid sequence all may have major importance. Indeed, previous studies of collagens from different tissues of a single species have demonstrated varying contents of lysine and hydroxylysine and recent studies in this laboratory have shown that the amount of 3-hydroxyproline also is not constant. 3-Hydroxyproline is a recently discovered imino acid related to the more common 4-hydroxyproline. These two imino acids may occupy interchangeable positions in the amino acid sequence.

The mechanism by which collagen may function to initiate and control calcification is not known. One approach to the problem is to study proteins which calcify as part of an abnormal process. One of these is elastin as found in aorta. In a recent study, an in vitro system, employing rat aorta and serum, was developed. Since it is known that elastin in aorta is calcified in the atheromatous lesions found in atherosclerosis, the in vitro system may closely parallel the disease state. The independent study of experimental variables such as inhibitors and activators, levels of calcium and phosphorus, and chemical modification of the elastin can be evaluated. For example, the observation that the deposition of mineral (apatite) is confined to elastin even though collagen fibrils are present, demonstrates that the protein matrix is only one part of a calcifying system. Other regulatory factors are present. In this respect, the presence of a lag phase indicates that inhibitors are present in serum. A further observation is that the presence of certain amino acids affects the rate of mineralization.

CALCIFICATION

The fundamentals of skeletal and dental tissue calcification are obscure in many details. During the past year experiments were conducted to evaluate the relation of fluoride to calcification and particularly its role in bone resorption. There has been increasing interest also in the possible role of fluoride in the treatment of osteoporosis, and some phases of our basic laboratory research on fluoride and bone metabolism have been motivated by this particular problem in bone diseases. Using the white rat as the laboratory animal, studies relative to calcification are attempting to evaluate fluoride effects in association with cortisone. Whereas hydrocortisone depressed urinary excretion of citrate, an important minor constituent of bone, fluoride had little or no such effect. With reference to bone-citrate per se large differences were found in the dental tissues of different species. The possibility exists that a variable citrate content affects bone and dental tissue crystallinity.

In parallel experiments, further evaluation was made of the effects of fluoride-induced changes on bone metabolism and bone-mineral crystallinity. Using X-ray diffraction analysis it was shown that fluoride incorporation improved bone apatite crystallinity. Bone and tooth mineral metabolism was investigated further in rats receiving 25 and 50 ppm of fluoride in their drinking water. In this case it was shown that the uptake of Ca^{45} and Sr^{89} in the tibia ends of the bones was decreased by these quantities of fluoride. Incisor tooth apatite, however, was not affected but there was a discrimination against Strontium vs. Calcium in the base of the incisor teeth.

In connection with the problem of osteoporosis, the influence of the level of calcium ingestion is of major importance. Thus additional studies were conducted with white rats receiving low and high calcium diets. The effect of fluoride was studied with these calcium-adequate and calcium-deficient diets and labeled Calcium as well as Strontium were administered during the course of the experiments. The relative absorption of Calcium vs. Strontium gives basic information on the exchange mechanisms of bone mineral. The results of this study suggest that there was a high skeletal uptake of Ca^{45} by rats receiving the Calcium deficient diets and this increased uptake was due principally to an increased rate of calcium exchange rather than to an accretion of calcium. Somewhat contrary to expectations, fluoride decreased slightly the uptake of radiocalcium. However there was an increase in total bone ash, due to fluoride. The facility for absorption of calcium in the intestinal tract is an additional important facet of adequate skeletal mineral metabolism. In further experiments it was shown that the intestinal absorption of Ca^{45} was greater than Sr^{89} as the level of calcium ingestion was increased. On a low calcium diet the absorption of both these isotopes was increased. Thus under normal conditions the organism adapted to an efficient utilization of a calcium deficient diet.

DENTAL CARIES - RELATION TO PHOSPHATES

Interest in the cariostatic role of phosphates continues with experimental studies being directed toward the role of organic phosphorus compounds. With remarkable consistency and significance, certain organic phosphorus compounds, i.e., phytin, sodium phytate, calcium phytate and β-glycerol phosphate were caries-preventive in the white rat. These results follow previous studies and similar results using inorganic phosphates. However, the mechanism of an anticaries effect of a soluble inorganic phosphate may indeed involve a less complicated action than that of an organic phosphorus compound. This significant anticaries action of both of these types of phosphates is highly provocative and may have practical applications in human caries control.

CONGENITAL MALFORMATION AND TERATOGENIC AGENTS

Using pregnant Sprague-Dawley rats, ACTH, cortisone, 17 hydroxycorticosterone, isoprotesinal, glutithionide, and thalidomide were investigated as teratogenic agents during specific periods of gestation. The treated animals

were killed on the 20th day of pregnancy and the young examined for malformations. This particular strain of rats proved particularly resistant to these treatments, thalidomide being the only agent which produced congenital malformations. When the latter drug was administered, 6 to 9% grossly malformed fetuses were found. Among the more common defects were malrotations of the hind limbs, hamartoma of the palate, accessory incisors and microsomia. Further studies revealed the presence of a subcutaneous cartilagenous type mass of tissue from middorsal region to the tail of the fetus.

In a parallel study, a special effort was made to evaluate the role of the hydrostatic pressure of the amniotic fluid, as well as amniocentesis (puncture of cell membrane) on congenital malformations. The principal defects following amniocentesis were cleft palate and limb deformities. It is of interest that cleft palate occurred only following amniocentesis on the 14th, 15th and 16th gestation day whereas limb deformities developed if amniocentesis was performed on any day except the 13th. A bacteriological study showed that infection was not a factor in teratogenesis by this procedure.

SALIVA AND SALIVARY GLANDS

Since salivary glands are reportedly enlarged and salivation is increased by isoproterenol hydrochloride, it was in our interest to investigate this observation with respect to susceptibility to experimental dental caries. Recently, investigators in this laboratory observed that this drug caused marked enlargement of the submaxillary and parotid glands in the rat, but did not affect the sublingual gland. Excessive salivation was also evident. When these animals were placed on a cariogenic diet, a 50% higher caries score resulted than in control rats. Interesting histological changes were observed in both the thyroid and salivary glands.

In other studies, biochemical data have revealed the presence of four times as much inorganic phosphate in human saliva as in rat saliva. However, human saliva was markedly lower in tyrosine, tryptophan and protein. Additionally, acid phosphatase activity of human saliva was 5 to 6 times as high as that of rat saliva whereas alkaline phosphatase was only 1/8th that of rat saliva. Finally, uric acid was found in the human but not in the rat saliva. These differences may foretell important differences in the dental caries susceptibility of these two species.

ENZYME CHEMISTRY

At the present time approximately 1000 enzymes have been described, and many of these have been studied intensively from physical, chemical, physiological and kinetic points of view. Despite years of effort by many teams of investigators in many institutions the precise structure of no single enzyme has been determined, and the mechanism of action of no enzyme has yet been established. It is the program of the Section on

Enzyme Chemistry to participate in the elucidation of enzyme structures and the nature of the catalytic process. Enzymes differ from each other in many essential respects; therefore, the work of the Enzyme Chemistry Section has included studies on different types of enzymes that permit various techniques to be applied, providing insight into the differences that exist as well as similarities.

Many enzymes contain metal ions built into the protein structure. Kinetic studies of carboxypeptidase B have described the complicated roles of different metals, zinc, cobalt and cadmium, in affecting the binding of various substrates to the enzyme and in determining the rates at which these substrates are hydrolyzed. The formation of carboxypeptidase B from its precursor has also been studied. Similar studies with procarboxypeptidase A of pig pancreas (instead of the conventional beef pancreas) have yielded an enzyme that differs in certain characteristics from the beef enzyme. In the activation process several intermediates and products have been detected by electrophoresis and ion exchange chromatography; one fraction that has enzyme activity has been obtained in crystalline form. Related studies in collaboration with NIAMD have resulted in the demonstration of an enzyme in pig kidney that hydrolyzes specifically glutamic and aspartic acid groups from proteins and peptides.

The enzyme aldolase is an essential catalyst in glycolysis, and is the subject of active investigation in many laboratories. In other laboratories the reversible dissociation of aldolase into thirds in acidic and urea solutions has been demonstrated recently. Parallel studies in this laboratory have shown similar dissociations to occur in alkaline solutions, and further dissociation into sixths has been indicated by ultracentrifuge studies at high pH values. The relationship between the fragments has been studied by chromatography and electrophoresis of the components separated by enzymatic digestion; these methods indicate that the thirds are identical to each other, but that the smaller subunits of each third are not identical. The kinetics of aldolase have been studied intensively with native and enzymatically modified preparations. In collaborative studies with Yale University, a partial reaction catalyzed by aldolase, in which there is no net chemical reaction, but merely exchange of one specific hydrogen atom with the hydrogen of the medium, has been shown to be greatly affected by modification of aldolase. The modified enzyme shows a primary isotope effect when deuterium or tritium are substituted for hydrogen in the substrate, whereas the native enzyme attacks all forms of hydrogen at the same rate. This change in relative rates of steps in the catalytic process is paralleled by changes in the rate of exchange of products of the net reaction into the substrate. The kinetic and structural studies can be combined to permit the construction of a theoretical model of the enzyme, that is being examined by further experiments.

Vitamin B_{12} has recently been found to occur in a modified form in which it participates as a coenzyme for several enzymes. In collaboration with NHI, the mechanism of the conversion of the vitamin to the coenzyme has been described. A single enzyme has been shown to be responsible for the

replacement of a cyanide group by deoxyadenosine derived from ATP. A unique feature of this reaction is the liberation of inorganic tripolyphosphate from ATP. Vitamin B_{12} also participates in the formation of methionine from homocysteine. The role of vitamin B_{12} in transferring methyl groups from tetrahydrofolic acid to homocysteine with a bacterial enzyme has been established, and the influence of vitamin B_{12} on the analogous animal liver enzyme has been described.

A special low molecular weight form of nucleic acid, S-RNA, is known to participate in protein synthesis by accepting amino acids from specific amino acid activating enzymes and transferring them to the polypeptide chain being synthesized by ribosomes. The establishment of the structures of individual types of S-RNA is a major biochemical problem, since the specificity for reaction with the individual activating enzyme and also the specificity for transfer to the appropriate part of the protein (i.e., reading the genetic code written in messenger RNA) are built into the S-RNA molecule. Until now studies on the structure of this material have been hampered by the lack of an adequate method for separating the more than 20 types of S-RNA from each other.

In contrast to the empirical methods of separating nucleic acids, a specific method is being developed based on the reaction of amino groups of amino acids, with N-carboxyanhydrides. This method has been shown to result in the formation of an amino acid polymer that precipitates with any S-RNA molecules that are carrying amino acids, but not with "uncharged" S-RNA. Work is in progress to establish optimal conditions for quantitative separation of charged from uncharged S-RNA, and methods are being developed for the analysis of the component nucleotides of the precipitated nucleic acid.

CONTROL OF METABOLIC PROCESSES

An objective of this phase of our enzyme research is the study of levels of coenzymes in their relation to control of metabolic pathways. In addition, abnormal products of metabolism were studied in relation to alterations in metabolism controls as may be caused by chemicals and disease products. Thus the levels of the nicotinamide nucleotide coenzymes were found to be present at high levels in several species of bacteria with high metabolic rates. These coenzymes also occur at high levels in mammalian liver and spleen where the metabolic rate is high. They are present in lower concentrations in soft and hard structural tissues where the metabolic rate is correspondingly low. Apparently the nicotinamide nucleotide coenzymes thus have a significant relation to metabolic activity in the body organs and tissues.

The glycolytic action of a rat-cariogenic streptococcus may possibly serve to explain a relation of this organism to dental caries etiology. Since it has been shown that sodium bisulfite inhibits experimental dental caries, it is of interest to study the effect of this chemical on the glycolytic activity of this particular streptococcus. Sodium bisulfite decreases the

amount of lactic acid produced by this organism from glucose with a subsequent increase in some of the more neutral products. This effect has not been found to follow the known alternate pathways of glycolysis, where products other than lactate are formed by microorganisms.

Serial No. NIDR-29
1. Biochemistry
2. Enzyme Chemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1962

Part A

Project Title: Protein and Amino Acid Chemistry. I. Proteolytic Enzymes: Chemical and Molecular Studies. II. Blood Clotting: Molecular Mechanisms.

Principal Investigator: Dr. J. E. Folk

Other Investigators: Dr. E. C. Wolff

Cooperating Units: Section of Physical Biology, NIAMD
Section of Histochemistry, NIAMD

Man Years (calendar year 1962):

Total:	3 1/4
Professional:	1 1/4
Other:	2

Project Description:

Objectives:

I. (a) Specificity and mechanism of action of the carboxypeptidases A and B.
(b) Purification and studies of the activation of the zymogen of carboxypeptidase A.
(c) Biochemical and histochemical studies of aminopeptidase A.

II. Mechanism of the thrombin-induced conversion of fibrinogen to fibrin.

Methods Employed:

I. (a) Kinetic studies are being continued with carboxypeptidase B. Chromatographic and electrophoretic procedures have permitted the separation of several active species of porcine carboxypeptidase A. A study of the relative kinetic properties of these active enzymes is underway.

(b) Conventional methods of protein separation as well as Sephadex and cellulose chromatography have been used in the purification of procarboxypeptidase A from porcine pancreas tissue.

(c) In attempts to purify aminopeptidase A, numerous protein separation procedures have been utilized including hydroxyapatite chromatography and preparative electrophoresis. The preparation of several glutamyl and aspartyl peptides is underway in a further effort to define the specificity of this enzyme.

II. Certain portions of Peptide B of fibrinogen are in the final stages of synthesis and are being compared to the fragments from the naturally occurring peptide. The modified Peptides A and B of fibrinogen are being tested for bradykinin-stimulating activity.

Major Findings:

I. (a) A number of kinetic parameters of carboxypeptidase B have been determined and have given a good deal of information concerning the mode of action of this enzyme. These data support an hypothesis of the formation of an amino derivative of the enzyme transiently formed during hydrolyses of substrates.

Three electrophoretically separable forms of carboxypeptidase A have been prepared from pig pancreas and have been designated α_1, α_2 and β. One form, α_1, has been crystallized. Preliminary studies indicate that the specificities of these three active forms are identical.

(b) Procarboxypeptidase A has been partially purified from pig pancreas gland. Studies of its mode of activation have shown that it is converted by trypsin to α_1, and then to α_2 but not to β carboxypeptidases A.

(c) Aminopeptidase A has been purified approximately 200-fold from porcine kidney cortex. The enzyme is activated by calcium at concentrations above 0.01 M and removes only glutamic and aspartic acids from the amino-terminal end of peptide chains.

II. Desulfated and trypsin as well as chymotrypsin-digested peptide B are extremely active in potentiating the physiological action of bradykinin.

Significance to Dental Research:

I. Understanding of the mechanism of action and specificity of proteolytic enzymes, the use of these enzymes for protein and enzyme modification and structure studies, and the knowledge of the particular functions and interrelationships of these enzymes contribute to the organization and understanding of basic biochemical processes.

II. A knowledge of the primary structures, physiological changes

and effects, and the basic mechanisms involved in the fibrinogen-fibrin transition may contribute to a systematic pharmacological and clinical approach to the correction of certain blood clotting abnormalities.

Proposed Course of Project:

I. Study of the structural relationships, enzymatic activities and physical characteristics of the several active forms of carboxy-peptidase A is underway. It is felt that these studies will give insight into the mode of action of this enzyme.

Attempts to further purify aminopeptidase A are underway. This enzyme promises to be a useful tool in protein structure and modification studies.

II. Studies designed to determine the sequence of amino acids necessary for the physiological effects noted with Peptide B are underway. Synthesis of further peptide fragments with possible activity is proposed.

Part B included Yes

PHS-NIH
Individual Project Report
Calendar Year 1962

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Glenner, G. G., McMillan, P. J. and Folk, J. E. A Mammalian Peptidase Specific for Hydrolysis of N-Terminal α-L-Glutamyl and Aspartyl Residues. Nature 194:867, June 1962.

Glenner, G. S., Folk, J. E. and McMillan, P. J. Histochemical Demonstration of a Gamma Glutamyltranspeptidase-like Activity. J. Histochem. and Cytochem. 10:481, July 1962.

Gladner, J. A., Murtaugh, P., Folk, J. E. and Laki, K. Nature of Peptides Released by Thrombin. Ann. New York Acad. of Sciences. In press.

Wolff, E. C., Schirmer, E. W. and Folk, J. E. The Kinetics of Carboxypeptidase B Activity. I. Kinetic Constants. J. Biol. Chem. 237:3094, Oct., 1962.

Folk, J. E., Wolff, E. C. and Schirmer, E. W. The Kinetics of Carboxypeptidase B Activity. II. Kinetic Constants of the Cobalt and Cadmium Enzymes. J. Biol. Chem. 237:3100, Oct. 1962.

Folk, J. E., Wolff, E. C., Schirmer, E. W. and Cornfield, J. The Kinetics of Carboxypeptidase B Activity. III. Effects of Alcohols on the Peptidase and Esterase Activities; Kinetic Models. J. Biol. Chem. 237:3105, Oct. 1962.

Honors and Awards Relating to this Project:

None

Serial No. NIDR-30
1. Biochemistry
2. Enzyme Chemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1962

Part A

Project Title: Enzyme Structure and Mechanism of Action

Principal Investigator: Dr. A. H. Mehler

Other Investigators: None

Cooperating Units: Yale University - Dr. I. A. Rose

Man Years (calendar year 1962):

Total:	2 3/4
Professional:	1
Other:	1 3/4

Project Description:

Objectives:

To determine the primary structure of the enzyme aldolase; to determine the nature of linkages between enzyme and substrate; to determine the factors that influence catalytic activity; and to ascertain the nature of the catalytic process.

Methods Employed:

The kinetic properties of aldolase are determined by spectrophotometric and radioactive isotope methods. These assays are done with native enzyme from various sources and with altered enzyme preparations. The structure of the enzyme is determined by ultracentrifugal, chromatographic, electrophoretic and polarimetric procedures. End groups and peptides are determined by chemical and enzymatic methods, including the use of specific chemical derivatives, chromatography and electrophoresis.

Major Findings:

The partial reactions through which aldolase accomplishes its overall catalytic reaction have been examined by means of several types of exchange reactions. It was found that a step that is not rate-limiting in the native enzyme from muscle becomes rate-limiting after alteration with carboxypeptidase A. The new rate-limiting step shows extreme discrimination against the

heavy isotopes of hydrogen whereas the native enzyme shows no discrimination. Native liver aldolase behaves like a partially altered muscle enzyme, and is further altered by carboxypeptidase A. Rates of exchange of triose phosphates with fructose diphosphate are unequal in the equilibration catalyzed by native aldolase; the ratio is exaggerated by a 10-20 fold decrease in the rate of equilibration of dehydroxyacetone phosphate following carboxypeptidase treatment. These results have led to an elaboration of the model of aldolase previously developed from kinetic studies.

End group analysis and ultracentrifugation of denatured enzyme have shown that aldolase is composed of at least three subunits. Analysis of the peptides obtained by the "fingerprint" method supports the concept that the enzyme is composed of three identical components, and that these are not composed of smaller identical fragments.

Significance to Dental Research:

These studies are part of a program to elucidate the basic nature of enzymes. Since all biological processes are fundamentally enzymatic, it is necessary to understand the nature of enzymes in order to obtain an understanding of normal and pathological biological processes.

Proposed Course of Project:

All of the approaches mentioned above will be continued to define additional structural features and catalytic properties of aldolase. Other enzymes and related materials from various sources will be investigated as part of a program to determine the chemical basis for enzyme specificity and catalytic properties.

Part B included Yes

PHS-NIH
Individual Project Report
Calendar Year 1962

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Mehler, A. H.: Kinetic Properties of Native and Carboxypeptidase-altered Rabbit Muscle Aldolase. J. Biol. Chem. In press.

Mehler, A. H., and Bloom, B.: Interaction Between Rabbit Muscle Aldolase and Dihydroxyacetone Phosphate. J. Biol. Chem. In press.

Mehler, A. H.: Phenolytic Oxygenases in The Oxygenases (O. Hayaishi, Ed.) Academic Press, New York, 1962.

May, Everette L., Millican, R. Carl, and Mehler, Alan H.: Synthesis of Ring C^{14}-Labeled Anthranilic and 3-Hydroxyanthranilic Acid. J. Org. Chem., 27, 2274 (1962).

Honors and Awards relating to this project:

None

Serial No. NIDR-31
1. Biochemistry
2. Protein Chemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1962

Part A

Project Title: The Molecular Basis of Connective Tissue Metabolism and Function.

Principal Investigator: Dr. G. R. Martin

Other Investigators: Dr. K. A. Piez, Dr. E. Schiffmann, Dr. S. E. Mergenhagen, Dr. H. Fullmer and Dr. H. Bladen.

Cooperating Units: Harvard School of Dental Medicine, Dr. Paul Goldhaber.

Man Years (calendar year 1962):

Total:	4
Professional:	1
Other:	3

Project Description:

Objectives:

I. To study the molecular basis of connective tissue disorders, and the level, metabolism and structure of various connective tissue components in normal animals and in experimental conditions such as lathyrism, and scurvy and in inflamed tissue.

II. To study the organic matrix in calcification and the factors affecting deposition of mineral in hard tissues.

Methods Employed:

Usual laboratory procedures.

Major Findings:

I. The incorporation of glycine-C^{14} into the subunits of skin collagen was followed in normal and lathyritic rats. Label appears first and at the same rate in the primary subunits, $\alpha 1$ and $\alpha 2$, and later in $\beta 1$ and $\beta 2$. This is explained by the fact that $\beta 1$ and $\beta 2$ are derived from $\alpha 1$ and $\alpha 2$ by intramolecular crosslinking. In lathyritic animals the formation of $\beta 1$ and

β2 is markedly reduced though the synthesis of collagen proceeds. This defect is evident long before the appearance of the gross symptoms of lathyrism. In addition, the action of a lathyrogen on bone grown in tissue culture has been demonstrated for the first time. In this system, the lathyrogen has its greatest effect on newly formed collagen by preventing the conversion of soluble collagen to an insoluble matrix. These findings are consistent with the suggestion that the lack of intramolecular crosslinks affects the integrity of connective tissue through a loss of fibril stability resulting in the gross symptoms characteristic of lathyrism.

II. Following a lag period, rat aortas incubated in rat serum in vitro accumulated substantial quantities of calcium and phosphate. Examination of the tissue by X-ray diffraction, microradiography, electron diffraction, and electron microscopy indicated that the calcium-phosphate phase which formed was hydroxyapatite and that the crystals were localized almost exclusively in elastin. Selective elimination of various components of the aorta with proteolytic enzymes indicated that the presence of elastin was required for mineralization. Collagen fibers did not appear to be required for the initiation of calcification, nor did they seem to undergo appreciable calcification in the time periods studied. Analysis of the initial lag period suggested that at least two changes occurred in serum prior to the mineralization of this tissue. Inhibitors of the reaction were destroyed, and the level of dialysable calcium was elevated due to its release from serum protein.

III. Studies on parathyroid hormone-induced bone resorption have been initiated. Calvaria, maintained in culture, respond to parathyroid hormone only after a lag of 24 hours. At this time substantial quantities of calcium and phosphate are mobilized from the bone and appear in the media. Analysis of the products of bone metabolism demonstrate a close correlation between bone resorption and citrate production.

Significance to Dental Research:

Since collagen is an important constituent of the hard and soft tissues of the oral cavity, a clarification of the events involved in its formation, calcification and destruction may contribute to our understanding of normal and pathological conditions in which collagen has a role.

Proposed Course of Project:

I. Continued study of collagen metabolism and mechanism of collagen synthesis.

II. Studies of calcification and resorption in bone maintained in tissue culture.

Part B included Yes

PHS-NIH
Individual Project Report
Calendar Year 1962

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Schiffmann, E., and Martin, G. R.: In Vitro Mineralization of Rat Aorta in Serum. Nature 194, 189-190 (1962).

Martin, G. R., Piez, K. A., and Lewis, M. S.: The Incorporation of (^{14}C) Glycine into the Subunits of Collagen from Normal and Lathyritic Animals. Biochim. Biophys. Acta. In press.

Martin, G. R., Schiffmann, E., Bladen, H. A., and Nylen, M.: Chemical and Morphological Studies on the In Vitro Calcification of Aorta. J. Cell Biol. In press.

Mergenhagen, S. E., Martin, G. R., and Schiffmann, E.: Studies on the Endotoxin of the Group C Meningococcus. J. Immunol. In press.

Bladen, H. A., and Martin, G. R.: Preferential Mineralization of Elastin in a Matrix Also Containing Collagen. Proc. of the Fifth Inter. Congress for Electron Microscopy, QQ 5, Academic Press, New York (1962).

Martin, G. R., and Goldhaber, P.: Action of Aminoacetonitrile on Bone in Tissue Culture. Biochim. Biophys. Acta. In press.

Rizzo, A. A., Martin, G. R., Mergenhagen, S. E., and Scott, D. B.: Mineralization of Bacteria. Science 135, 439-441 (1962).

Honors and Awards relating to this project:

None

Serial No. NIDR-32
1. Biochemistry
2. Protein Chemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1962

Part A

Project Title: Physical Chemistry of Proteins

Principal Investigator: Dr. M. S. Lewis

Other Investigators: Drs. J. E. Folk, L. F. Hass, G. R. Martin, and K. A. Piez

Cooperating Units: NIAMD, Laboratory of Physical Biology

Man Years (calendar year 1962):

Total:	1-1/2
Professional:	1
Other	1/2

Project Description:

Objectives:

The purpose of this project is to study the physical chemistry of collagens and other proteins, particularly with respect to the functional relationship of molecular weight, dimensions, and structure.

Methods Employed:

Analytical ultracentrifugation was used for the determination of sedimentation coefficients and molecular weights; optical rotation for studies on helical content of native and denatured proteins; and viscometry for studies on molecular dimensions.

Major Findings:

(1) The molecular weights of native G-actin and of a modified form of this protein in various solvents have been determined. These data, in combination with data on the sedimentation coefficients, optical rotation, and intrinsic viscosity have made it possible to postulate a structural model for this protein and results indicate one of the roles of the prosthetic ATP group in the polymerization of actin.

(2) Ultracentrifugal studies on muscle aldolase exposed to alkaline buffers indicate that the molecule apparently remains intact up to relatively high pH values, then probably dissociates into three dimeric units, which undergo further rapid dissociation into six monomeric subunits at higher pH values. It also appears that the molecule undergoes progressive unfolding at least up to the point of dissociation.

(3) The molecular weight of the 3S component of myosin has been determined in 5 M guanidine, in 8 M urea, and in normal aqueous solvents. The relationship of these weights indicates a three stranded structure for myosin similar in some respects to collagen.

(4) The molecular weights of unfractionated porcine carboxypeptidase A and the purified α-2 fraction have been determined. Work is in progress on the purified α-1 fraction.

(5) Improvements have been effected in the equilibrium technique for the determination of molecular weights.

Significance to Dental Research:

Studies on the molecular structure of collagen are particularly significant because of the unique function of collagen as the major structural protein of connective tissue and its role in calcification. The proteins myosin and actin occupy a similar position relative to muscle tissue. The enzymes aldolase and carboxypeptidase A are typical enzymes of importance to all branches of medical science and basic biochemistry.

Proposed Course of Project:

(1) Studies are continuing on the structure and function of the various collagens and their subunits.

(2) Additional studies will be carried out on other proteins of significance in the general area of protein structure and function.

Part B included Yes

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Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Lewis, M. S., Maruyama, K., Carroll, W. R., Kominz, D. R., and Laki, K. Physical Properties and Polymerization Reactions of Native and Inactivated G-Actin. Biochemistry, in press.

Martin, G. R., Piez, K. A., and Lewis, M. S. The Incorporation of (^{14}C) Glycine with the Subunits of Collagen from Normal and Lathyriti Rats. Biochim. Biophys. Acta, in press.

Piez, K. A., Eigner, E. A. and Lewis, M. S. The Chromatographic Separation and Amino Acid Composition of the Subunits of Several Collagens. Biochemistry, in press.

Kominz, D. R., and Lewis, M. S. The 3S Component of Myosin.in Conference on Biochemistry of Muscle Contraction. Dedham, Mass., May 1962. John Gergely, editor. To be published by Little, Brown and Company.

Kominz, D. R., Maruyama, K., Levenbook, L. and Lewis, M. S. Tropomyosin, Myosin, and Actin from the Blowfly Phormia Regina. Biochim. Biophys. Acta 63:106 (1962).

Honors and Awards relating to this project:

None

Serial No. NIDR-33
1. Biochemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1962

Part A

Project Title: Biochemical and Biophysical Studies of Calcification

Principal Investigator: Dr. R. C. Likins

Other Investigators: See subprojects

Cooperating Units: The Roosevelt Hospital, New York, N. Y.
The Pennsylvania State University, University Park, Pa.

Man Years (calendar year 1962):
Total: 2-1/2
Professional: 1-1/2
Other: 1

Project Description:

Subproject A: (With Dr. J. Menczel, NIH Visiting Associate, Hebrew University and Hadassah Medical School, Jerusalem, Israel, and Dr. H. Schraer, Visiting Scientist, Pennsylvania State University, University Park, Pa.)

Objectives:

The effect of fluoride on the crystallinity of bone and tooth mineral and the comparative skeletal metabolism of strontium^{89} and calcium^{45}.

Methods Employed:

Rats were given drinking water containing 0, 10, 25, 50 and 100 ppm fluoride for one year, then injected with strontium^{89} and calcium^{45}. The bones and teeth were analyzed radiochemically, and studied by X-ray diffraction line broadening.

Major Findings:

X-ray diffraction showed that fluoride improved bone apatite crystallinity in the direction of the a axis but not the c axis. Fluoride did not affect incisor apatite crystallinity. Uptake of calcium^{45} and strontium^{89} decreased in the tibia ends due to

Part A (continued)

25 and 50 ppm F. Strontium^{89} /calcium^{45} ratios were the same in tibia and serum. There was a discrimination against strontium in relation to calcium in the incisor teeth.

Significance to Dental Research:

Effect of fluoride on dental caries may be explained by crystal chemistry of hydroxyapatite.

Proposed Course of Project:

A study of the effect of carbonate content on bone and tooth crystallinity.

Subproject B: (With Dr. J. Menczel and Dr. R. Schraer, Department of Biophysics, Pennsylvania State University, University Park, Pa.)

Objectives:

The effect of fluoride on experimentally induced osteoporosis.

Methods Employed:

Chemical and X-ray diffraction analyses of femurs of rats receiving a low and high calcium diet with and without fluoride supplementation. The uptake of calcium^{45} by ends and shafts of tibia was determined.

Major Findings:

Both the ends and shafts of rats fed the calcium deficient diet, with and without fluoride, contained significantly more radiocalcium than the controls. Thus the metabolism of calcium at the skeletal level was profoundly altered by a long-term ingestion of a calcium deficient diet. The level of calcium intake did not affect bone crystallinity as determined by X-ray line broadening analysis. The results suggest that the higher skeletal uptake of calcium^{45} by the calcium deficient rats is principally due to an increased rate of exchange, rather than to bone accretion. Fluoride decreased slightly the uptake of radiocalcium and increased the bone ash weight.

Significance to Dental Research:

It has been reported that the calcium balance in osteoporosis can be improved by the administration of large doses of sodium fluoride. There is also evidence that the prolonged ingestion of large amounts

Part A (continued)

of fluoride in the drinking water may counteract osteoporotic changes in humans. This effect of fluoride in osteoporosis may due to structural and morphological changes in the mineral phase of bone. Bone resorption may be affected since crystallinity of bone apatite improves with an increase in fluoride.

Proposed Course of Project:

Continued studies will investigate skeletal exchange and accretion in experimentally induced osteoporosis.

Subproject C: (With Dr. J. Menczel)

Objectives:

The effect of Ca and Sr intake on the absorption and comparative metabolism of calcium^{45} and strontium^{89}. Effect on bone crystallinity and osteoporosis.

Methods Employed:

Rats were intubated with a liquid diet containing two different amounts of calcium plus trace amounts of calcium^{45} and strontium^{89}. At various time intervals the absorption, excretion, and skeletal deposition of the two isotopes were determined.

Major Findings:

The absorption of Ca^{45} was greater than Sr^{89} at both levels of calcium intake. On the low calcium diet the absorption of the two isotopes increased. One hour after the start of the experiment the serum Sr^{89}/Ca^{45} ratio was the same for both diets. At the end of two and four hours the Sr^{89}/Ca^{45} serum ratios were higher in animals receiving the low calcium diet. Proportionately more of the absorbed Sr^{89} was incorporated in bones of rats fed the calcium deficient diet.

Significance to Dental Research:

The dietary calcium intake and metabolism with respect to the absorption, excretion, and skeletal incorporation of Sr may be intimately related to bone and tooth development and function.

Proposed Course of Project:

This research may be extended into a study of the effect of calcium intake in relation to age.

Part B included Yes

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Part B Honors, Awards and Publications

Publications other than abstracts from this project:

Rubin, M yer, Likins, Robert C., and Berry, Elmer G. On the Validity of Radiocarbon Dates from Snail Shells. J. Geol. 70, December 1962.

Menczel, J., Posner, A. S., Schraer, H., Pakis, G., and Likins, R. C. Comparative Fixation of Sr89 and Ca45 by Calcified Tissues as Related to Fluoride Induced Changes in Crystallinity. Proc. Soc. Exptl. Biol. Med. 110: 609-613, July 1962.

Arnold, Francis A., Likins, Robert C., Russell, A. L., and Scott, David B. Fifteenth Year of the Grand Rapids Fluoridation Study. Accepted for publication in the J. Am. Dent. Assoc.

Menczel, J., Schraer, R., Pakis, G., Posner, A. S., and Likins, R.C. Effect of a Low Calcium Diet on Bone Crystallinity and Skeletal Uptake of Ca^{45} in Rats. Accepted for publication in the Proc. Soc. Exptl. Biol. Med.

Likins, R. C., Berry, E. G., and Posner, A. S. Comparative Fixation of Ca and Sr by Snail Shell. Accepted for Publication in Proc. N. Y. Acad. Sciences.

Honors and Awards relating to this Project:

None

Serial No. NIDR-34
1. Biochemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1962

Part A

Project Title: The Cariostatic Effect of Phosphate.

Principal Investigator: Dr. F. J. McClure

Other Investigators: Dr. C. J. Donnelly

Cooperating Units: None

Man Years (calendar year 1962):

Total:	4 1/4
Professional:	1
Other:	3 1/4

Objectives:

To evaluate the role of phosphate compounds as cariostatic agents.

Project Description:

Subproject A:

Effect of organic and inorganic phosphates on experimental rat caries.

Methods Employed:

Usual experimental design for caries assessment in rats.

Major Findings:

The effects of different phosphorus compounds on experimental dental caries were as follows: as added to cariogenic diets containing essentially dry bread and glucose, dental caries was significantly inhibited by three orthophosphates, i.e. $Ca(H_2PO_4)\cdot H_2O$, $Ca_3(PO_4)_2$ where salt was present in the diet, and $(NH_4)_2HPO_4$; and by two metaphosphates, i.e. $Na(PO_3)_n$ and $Na(PO_3)_6$. The results with the orthophosphates confirm previous results. Victamide, an ammonium P_2O_5 complex as well as $(KPO_3)_n$ were not significantly cariostatic. The following organic phosphates were unusually cariostatic: phytin, sodium phytate, calcium phytate, and β-glycerol phosphate. The

results with the organic phosphates add a new and provocative perspective to the possible mechanism of the phosphate action on experimental dental caries.

Subproject B:

Effect of phosphate in a dentifrice.

Objectives:

To determine the cariostatic effect of a dentifrice containing 10% soluble Na_2HPO_4 and 30% tricalcium phosphate vs. a placebo dentifrice containing no phosphate ingredient, i.e. 40% chalk in the abrasive.

Methods Employed:

Approximately 1000 boys, age 8-18 years, living in a self-contained School for Boys, are divided into two comparable groups: one receiving the phosphate dentifrice, the other the placebo. Initial dental examinations, including X-rays, have been completed. It is planned to continue the study three years with regular yearly dental examinations.

Major Findings:

None

Significance to Dental Research:

The use of phosphates has potential value in the control of human dental caries. The cariostatic effect of a number of both organic and inorganic phosphates on experimental animal caries seems quite conclusively documented.

Proposed Course of Projects:

Further studies with experimental rats to evaluate other organic phosphates.

Part B included Yes

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Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Van Reen, R., König, K. G., Ostrom, C. A. and McClure, F. J. Evaluation of Dental Caries in the Rat; a Comparison of Grinding and Slicing Techniques in Two Strains of Rats Fed a Purified Diet of High Cariogenic Capacity with Orthophosphate Supplements. Arch. oral Biol. 17:481-489, 1962.

Fluoride Drinking Waters, F. J. McClure, Editor. U. S. Dept. of Health, Education, and Welfare, Public Health Service, National Institute of Dental Research, Bethesda, Md., PHS Publication 825, 1962, 636 pages.

McClure, F. J. Further Studies on the Cariostatic Effect of Organic and Inorganic Phosphates. J. Dent. Research. In press.

Honors and Awards Relating to this Project:

None

Serial No. NIDR-35
1. Biochemistry
2. Protein Chemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1962

Part A

Project Title: Analytical and Structural Aspects of Collagen and Other Proteins in Relation to Function.

Principal Investigator: Dr. K. A. Piez

Other Investigators: Dr. G. Martin, Dr. M. S. Lewis

Cooperating Units: None

Man Years:

Total:	2 1/2
Professional:	1
Other:	1 1/2

Project Description:

Objectives:

It is the long-range purpose of this project to examine collagens and proteins of related interest with respect to their chemical and physical nature and to correlate function and structure in normal and pathological conditions. The immediate problems relate to (1) the separation and characterization of the subunits of collagen, (2) intramolecular crosslinking of collagen as a function of age, and (3) the characterization of noncollagenous, calcified proteins.

Methods Employed:

Usual.

Major Findings:

The subunit structure of collagen from a number of vertebrate species and different tissues of the same species has been investigated. The structure of the collagen molecule can be represented by the formula $(\alpha 1)_2\alpha 2$ where $\alpha 1$ and $\alpha 2$ represent related subunits of different amino acid composition. After denaturation $\alpha 1$ and $\alpha 2$ can be separated by chromatography and studied separately. Intramolecular crosslinking produces the molecular structures $\alpha 1\beta 2$, $\alpha 2\beta 2$, and γ where $\beta 1$ and $\beta 2$ represent dimers of $\alpha 1$ and $\alpha 2$

($\beta 1 = \alpha 1\text{-}\alpha 2$, $\beta 2 = \alpha 1\text{-}\alpha 1$) covalently linked and γ represents a trimer ($\alpha 1\text{-}\alpha 1\text{-}\alpha 2$) also covalently linked.

Collagens from rat skin and tendon, guinea pig skin, fish swim bladder (ichthyocol), and dogfish skin have been studied. Each of these collagens has a characteristic amino acid composition. The portions extractable with neutral salt solution and dilute acetic acid, which represent, respectively, newly made and somewhat older collagen, show characteristic distributions of the several collagen structures. For example, salt-extracted collagen from rat skin is approximately 75% $(\alpha 1)_2\alpha 2$ indicating a very low degree of intramolecular crosslinking whereas acid-extracted collagen from rat tail tendon is approximately 90% $\alpha 1\beta 1$ and $\alpha 2\beta 2$. Dogfish collagen is nearly all crosslinked and contains an appreciable amount of γ .

It is apparent that intramolecular crosslinking proceeds at different rates and to different extents in different tissues and animals. These results, together with the evidence obtained from lathyritic animals (where the crosslinking does not occur), indicate that crosslinking is an important maturation process which is necessary to the proper function of the tissue.

In addition to the differences in lysine and hydroxylysine contents of collagen from different tissues of the same species which has been previously reported, it has now been found that the content of 3-hydroxyproline (a recently discovered imino acid) is also not constant. For example rat skin collagen contains about 1 residue per 1000 while rat tendon collagen contains about 4 residues per 1000.

It is possible that these apparently minor differences between collagens from different tissues of the same animal are critically related to function.

Significance to Dental Research:

(1) Collagen is a major structural protein and appears to play a specific role in certain metabolic processes. An understanding of its molecular structure is therefore basic to an understanding of many normal and pathological conditions. For example, the elucidation of the crosslinking mechanism between subunits of the collagen molecule appears to explain some of the changes that occur in connective tissue with age. (2) In determining the role which a protein matrix plays in calcification, a knowledge of the properties of the matrix is a necessity. In particular, a comparison of calcified collagens and noncollagenous proteins may help to elucidate the mechanism by which they exert an effect on the initiation and progression of calcification.

Proposed Course of Project:

Studies will continue to further characterize the subunits of collagen. It is hoped to employ selective degradation to obtain smaller units which will be more amenable to chemical studies. The comparison of collagens from different tissues and different species will be broadened to help elucidate those aspects of chemistry and structure which are important to function.

The comparative study of noncollagenous calcified proteins from invertebrate species and enamel, which has not been actively pursued this year, will be continued next year.

Part B included Yes

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Individual Project Report
Calendar Year 1962

Part B. Honors, Awards and Publications

Publications other than abstracts from this project:

Piez, K. A. Chemistry of the Protein Matrix of Enamel. In Fundamentals of Keratinization. Ed. by E. O. Butcher, American Association for the Advancement of Science, Washington, D. C. Chap. II, p. 173-187, 1962.

Piez, K. A. Continuous Scintillation Counting of Carbon-14 and Tritium in the Effluent of the Amino Acid Analyzer. Anal. Biochem. In press.

Martin, G. R., Piez, K. A. and Lewis, M. S. The Incorporation of (^{14}C) Glycine into the Subunits of Collagen from Normal and Lathyritic Rats. Biochim. Biophys. Acta. In press.

Piez, K. A., Eigner, E. A. and Lewis, M. S. The Chromatographic Separation and Amino Acid Composition of the Subunits of Several Collagens. Biochem. In press.

Piez, K. A. The Amino Acid Chemistry of the Matrices of Some Calcified Tissues. New York Academy of Sciences. In press.

Honors and Awards relating to this Project:

None

Serial No.NIDR-36
1. Biochemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1962

Part A

Project Title: Metabolic Controls

Principal Investigator: Dr. S. A. Narrod

Other Investigators: Dr. H. V. Jordan

Cooperating Units: None

Man Years (calendar year 1962):

Total:	1 1/2
Professional:	1
Other:	1/2

Project Description:

Objectives:

(1) To investigate the levels of nicotinamide nucleotide coenzymes and their relation to control of metabolic pathways.

(2) To study some abnormal products of metabolism in relation to alterations in control as caused by chemicals and disease.

Methods Employed:

(1) Standard fluorescent, enzymic, and chemical analyses are being developed to measure oxidized and reduced nicotinamide coenzymes in microorganisms under varying conditions.

(2) (a) A sensitive enzymic and chemical assay is being developed for the measurement of ethanolamine phosphate in biological tissues. The assay is based on enzymic conversion of ethanolamine to glycolaldehyde as described previously (see project report NIDR-9, 1957, Narrod).

(b) Alteration of metabolic products of a rat cariogenic microorganism by sodium bisulfite is being studied using standard spectrophotometric and manometric procedures.

Major Findings:

(1) Preliminary investigation indicates that the nicotinamide nucleotide coenzymes can be extracted chemically from several different types of microorganisms without additional procedures for rupturing or disintegrating the cell walls. The levels of the nucleotides in these microorganisms are similar to those in mammalian liver and spleen while considerably lower quantities are present in soft and hard structural tissues. Of those organisms studied thus far, the aerobic class has slightly higher levels of these coenzymes.

(2)(a) The conversion of ethanolamine to glycolaldehyde by a pseudomonad type microorganism occurs by an induced enzymic system. A large scale production of the organism was developed and suitable enzyme purification procedures are under investigation. The enzyme was purified about 4-fold and found to be stable for at least 2 weeks when stored at 3° C or lower.

Conversion of the bis addition product of glycolaldehyde to a compound which can be measured spectrophotometrically at 550 mμ has resulted in an overall extremely sensitive assay for ethanolamine. Of several compounds tested, only ethanolamine appears to react.

(b) The addition of sodium bisulfite to the glucose fermentation medium of a cariogenic rat-streptococcus has been shown by Dr. Jordan to convert the metabolism from the homolactic products normally found in this species to more heterolactic products. Preliminary evidence indicates that alternate pathways of glycolysis associated with heterolactic fermentation are not present in this organism.

Significance to Dental Research:

Understanding the metabolic controls may aid in studies of aberrations of normal metabolism leading to dental malformations and periodontal diseases. Thus it is hoped that a new ethanolamine assay can be used to study ethanolamine phosphate, a compound reported to be eliminated in association with premature loss of teeth and various bone disorders. A study of altered patterns of metabolism induced by sodium bisulfite may give basic information on the etiology of dental caries.

Proposed Course of Project:

(1) Studies of the nicotinamide coenzymes in microorganisms will be extended to other metabolic types and eventually to other biological systems. Changes in levels of these coenzymes

following treatment by various substrates and drugs will also be investigated. If alterations in coenzymes are found to occur, their effect on other metabolic reactions will be examined.

(2)(a) By purification of the enzyme converting ethanolamine to glycolaldehyde, it is hoped that the test for ethanolamine and its phosphate derivative will be made rapid enough so that it can be used in patients with hypophosphatasia and periodontal diseases.

(b) Studies pertinent to sodium bisulfite and its effect on bacterial metabolism will continue.

Part B included No

Serial No. NIDR-37
1. Biochemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1962

Part A

Project Title: Biochemistry and Physiology of the Salivary Glands and Their Secretions.

Principal Investigator: Dr. I. Zipkin

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1962):
Total: 1 1/2
Professional: 1/2
Other: 1

Project Description:

Objectives:

To study basic components of parotid and submaxillary saliva and their relation to oral and systemic disease.

Methods Employed:

Normal human mixed saliva and mixed rat saliva were analyzed for protein, tyrosine, tryptophan, uric acid, citrate, alkaline and acid phosphatases. Serum of the rat was also analyzed for acid and alkaline phosphatases. Comparisons of the Na, K, Ca, and Mg and buffer capacity contents were taken from the literature.

Major Findings:

Human saliva contained four times as much inorganic phosphorus as rat saliva, but was markedly lower in tyrosine, tryptophan and protein. The acid phosphatase activity of human saliva was 5-6 times as high as that of rat saliva, but the alkaline phosphatase activity was only 1/8 that of rat saliva. In addition, human serum had only about 1/10 the alkaline phosphatase activity of rat serum. No uric acid could be found in the saliva of the rat.

Proposed Course of Project:

Other studies in progress are:

(a) In collaboration with Dr. King the effects of antarctic stress on the chemistry of the parotid saliva of Navy trainees is being investigated.

(b) The transport of antibodies to parotid saliva in response to various bacterial endotoxin antigens is being investigated in collaboration with Dr. Mergenhagen.

(c) We plan to devote our major effort to a study of the polysaccharides and mucoproteins of saliva in their relation to such diseases of the mucous secretions as cystic fibrosis. These studies will be approached from both an immunological as well as a biochemical viewpoint.

Significance to Dental Research:

These data as well as others recorded from the literature with respect to Ca, Mg, Na and K indicate that differences in chemical composition of the saliva of experimental species should be considered in the selection of an appropriate animal for the investigation of such oral conditions as caries and periodontal disease.

Part B included Yes

PHS-NIH
Individual Project Report
Calendar Year 1962

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Larson, R., Rubin, M., and Zipkin, I.: Frequency of Eating as a Factor in Experimental Dental Caries. Arch. Oral Biol. 7, 463-8 (1962).

Mergenhagen, S. E., Zipkin, I., and Varah, E.: Immunochemical Studies on an Oral Veillonella Endotoxin. J. Immunol. 88, 482 (1962).

Larson, R. H., Zipkin, I., and Fitzgerald, R. J.: The Effect of Dehydroacetic Acid and Tetracycline on Caries Activity and its Transmission in the Rat. J. Dent. Res. In press.

Jordan, H. V., Zipkin, I., Fitzgerald, R. J., and Berger, N. D.: Effect of Lathyrogenic Agents on Dental Caries in the Rat. J. Dent. Res. In press.

Zipkin, I., Hawkins, G. R., and Mazzarella, M.: The Tyrosine, Tryptophan and Protein Content of Human Parotid Saliva in Oral and Systemic Disease. Use of Ultraviolet Absorption Technics. Arch. Oral Biol. In press.

Zipkin, I., and Hawkins, G. R.: Species Specificity in Salivary Biochemistry. J. Dent. Res. In press.

Honors and Awards relating to this project:

None

Serial No. NIDR-38
1. Biochemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1962

Part A

Project Title: Factors Affecting Calcification of the Skeletal and Dental Tissues.

Principal Investigator: Dr. I. Zipkin

Other Investigators: Dr. Jacob Menczel, Hadassah University Medical School, Jerusalem, Israel

Dr. Aaron Posner, Laboratory of Histology and Pathology, NIDR

Cooperating Units: Pennsylvania State University - Dr. Harold Schraer.
New York University Dental School - R. S. Gold.

Man Years (calendar year 1962):
Total: 3 1/2
Professional: 1/2
Other: 3

Project Description:

Objectives:

To study the role of fluoride and citrate in the calcification process in bones and teeth.

Subproject A:

Role of fluoride in bone resorptive processes.

Methods Employed:

Four groups of six rats respectively received hydrocortisone acetate (1 mg/day), 50 ppm F, hydrocortisone acetate plus 50 ppm F, and the hydrocortisone vehicle (control group). Urines and feces were collected throughout the 80-day study. Preliminary studies had established the maximum tolerable dose in the weanling rat for hydrocortisone at 1 mg/day. Histological studies had also demonstrated decreased trabeculation in the metaphysis at this dosage level. Urines have been analyzed for citrate, total nitrogen and hydroxyproline.

Major Findings:

Hydrocortisone depressed the urinary excretion of citrate to a marked degree, whereas fluoride had little or no effect in inhibiting citrate excretion. Neither hydrocortisone nor fluoride appeared to affect nitrogen excretion. The steroid markedly inhibited hydroxyproline excretion whereas fluoride alone slightly potentiated its excretion.

Proposed Course of Subproject A:

(a) In addition to citrate, nitrogen and hydroxyproline determinations on bones, teeth, urine and feces in Subproject A, Ca, P, Mg and F will also be determined in these tissues.

(b) In addition, an acute experiment on the interplay of fluoride and hydrocortisone in the resorptive process has been completed. 2.5 mg/day of hydrocortisone and 75 ppm F were administered. The bones will be analyzed for Ca, P, Mg, F, citrate, nitrogen and hydroxyproline.

Subproject B:

The citrate content of the dental tissue of various species.

Methods Employed:

Dentin and enamel of the teeth of the human, monkey, cow, dog, sheep, fox, pig, white rat, cotton rat, white mouse, field mouse, puma and hamster were analyzed for citrate.

Major Findings:

A surprisingly large difference was found in the concentration of the citrate in the dental tissues of various species. Thus the dentin of man contained about 0.9% citrate whereas the same tissue in the molar of the hamster contained only about 0.1%. The teeth of the pig, presumed to be similar embryologically to the human, contained only about 0.2% citrate in the dentin.

Proposed Course of Subproject B:

The effect of the widely varying citrate content of the dental tissues on their degree of crystallinity will be studied with the collaboration of Aaron S. Posner.

Significance to Dental Research:

The role of fluoride in the calcification process in bones and teeth is unknown. The studies presented here are designed to determine the sequence of events in the bone resorptive process and to determine the site of initial resorption, i.e., in the organic or inorganic matrix, and to determine the role of fluoride in this process. In addition, the interrelation of fluoride, citrate and hard tissue crystallinity is being investigated. These studies will be extended to the dental tissues to elucidate the interrelation of fluoride, citrate and enamel crystallinity and its relevance to the carious process.

Part B included Yes

Serial No. NIDR-38
1. Biochemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1962

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Zipkin, I., Posner, A. S., and Eanes, D. E.: The Effect of Fluoride on the X-ray Diffraction Pattern of the Apatite of Human Bone. Biochim. Biophys. Acta 59, 255-8 (1962).

Zipkin, I., Schraer, R., Schraer, H., and Lee, W. A.: The Effect of Fluoride on the Citrate Content of the Bones of the Growing Rat. Arch. Oral Biol. In press.

Schraer, H., Posner, A. S., Schraer, R., and Zipkin, I.: Effect of Fluoride on Bone Crystallinity in the Growing Rat. Biochim. Biophys. Acta. In press.

Honors and Awards relating to this project:

None

Serial No. NIDR-39
1. Biochemistry
2. Enzyme Chemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1962

Part A

Project Title: Studies on Soluble Ribonucleic Acid

Principal Investigator: Dr. Fred H. Bergmann

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1962)
Total: 1
Professional: 1
Other: none

Project Description:

Objectives:

Enzymatic reactions forming amino-acyl ribonucleic acid compounds have been described. The present work was primarily concerned with a chemical approach to the fractionation of individual soluble RNA species.

Methods Employed:

A variety of N-carboxy amino acid anhydrides were prepared by conventional techniques of organic chemistry. Isotope methods were used to determine amino-acyl RNA formation, and the reactions of these with N-carboxy anhydrides (NCA's).

Major Findings:

1. When valyl-1-C^{14}-SRNA was reacted with ξ-Carbobenzoxylysyl-NCA or phenylalanyl NCA, a large proportion of the added radioactivity was bound to the water-insoluble polymer formed during the reaction. The supernatant solutions from such reaction mixtures contained the bulk of the underivatized soluble RNA. This residual material accepted significantly reduced amounts of C^{14} valine, but essentially normal amounts of a control amino acid (C^{14} leucine). These results indicate that a specific reaction had occurred between amino-acyl S-RNA and the NCA. Cleavage of

compounds thus formed should result in considerable purification of a specific RNA species. A variety of reagents were investigated to accomplish this aim. Up to now, all procedures tried have resulted in failure to solubilize the derivatized S-RNA, or have inactivated control S-RNA. The difficulties encountered may reflect increased stability of the polypeptide-S-RNA bond, compared to amino acyl-S-RNA, and thus give some insight into the nature of the linkage between the amino acid and the RNA.

2. The possibility of employing the incorporation of amino acids into S-RNA as a technique in the determination of specific amino acids was briefly investigated. The proposed method would depend on isotope dilution. It is believed, on the basis of these studies, that such an application is feasible, would be reasonably simple and accurate, and would be sensitive in the microgram and submicrogram range.

Significance to Dental Research:

It is believed that studies of the type described here will lead to a more thorough understanding of the specificity of reactions that participate in protein synthesis. This should contribute to a more thorough understanding of biological processes at the molecular level.

Proposed Course of Project:

The nature of S-RNA will continue to be studied. An investigation of the specificity of an enzyme involved in the formation of the terminal nucleotide residues in S-RNA is under way.

Part B included No

Serial No. NIDR-40
1. Biochemistry
2. Enzyme Chemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1962

Part A

Project Title: Characterization of the Structure and Function of Muscle Aldolase by Physiochemical and Enzymatic Methods

Principal Investigator: Dr. L. F. Hass

Other Investigators: Dr. M. S. Lewis and Dr. A. H. Mehler

Cooperating Units: None

Man Years:

Total:	1
Professional:	1
Other:	None

Project Description:

Objectives:

1. To determine the molecular structure of muscle aldolase.

2. To correlate aldolase structure with function.

Methods Employed:

Conventional physical, chemical, and enzymatic techniques were used.

Major Findings:

1. Ultracentrifugal analyses of muscle aldolase exposed to highly alkaline buffers at room temperature have shown that the native molecule is rapidly dissociated into six monomeric subunits of apparently equal molecular weight. Similar analyses at slightly lower alkaline pH values indicate that dissociation probably proceeds through the formation of three dimeric intermediates which disrupt further to give the fundamental subunits.

Part A (continued)

2. Reconstitution studies on denatured aldolase have shown that after brief exposure to strong alkali, a major portion of the initial aldolase activity can be regained. Additional data indicate that the ability to reconstitute decreases as a function of the length of time aldolase is exposed to alkali and that after long periods of time no reactivation can be obtained. Since reconstitution was also found to be dependent on aldolase concentration, these experiments indicate that aldolase in alkali undergoes an initial, reversible, dissociation into relatively high molecular weight subunits which then irreversibly dissociate into monomeric species.

3. Polarimetric and spectrophotometric measurements indicate that when aldolase is exposed to a highly alkaline environment, gross molecular disorganization occurs.

4. Preliminary analyses indicate that aldolase probably terminates in prolylproline at the amino end(s) of the molecule. So far, attempts to quantitate these N-terminal amino acids have been unsuccessful.

5. Using classical chemical procedures, a method for the synthesis of alkyl diphosphates has been developed. Several diphosphoryl compounds have been synthesized and tested as inhibitors of the aldolase reaction.

Significance to Dental Research:

Characterization of the structure of aldolase will provide fundamental information about the chemical nature of enzymes, which is basic to an understanding of how enzymes function and how they may be influenced.

Proposed Course of Project:

1. Attempts will be made to disrupt the aldolase molecule by various methods. It is anticipated that these methods will provide a means for separating and characterizing the individual chains which combine to form the native molecule.

2. Physicochemical studies will be continued in an effort to gain further insight into the gross molecular organization of aldolase.

3. Enzymatic degradation procedures will be employed to determine their influence on aldolase activity.

Part B included No

Serial No. NIDR-41
1. Biochemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1962

Part A

Project Title: Prenatal Influences on Fetal Development

Principal Investigator: Dr. F. J. Kendrick

Other Investigators: Dr. C.T.G. King

Cooperating Units: None

Man Years (calendar year 1962):
Total: 2
Professional: 1
Other: 1

Project Description:

Objectives:

To study the effects of environmental factors upon the development of the rat fetus with particular reference to oral structures.

Methods Employed:

1. Puncture of fetal membranes by passing a hypodermic needle through the uterine wall and into the amniotic cavities of (a) intact rats whose stage of gestation varied from 13 to 18 days, inclusive, and (b) of adrenalectomized rats on the 15th day of gestation. In the pregnant Ajax mouse exogenous corticosterone induces cleft palate and hair lip in the fetuses. Adrenalectomy was performed in this series of animals to eliminate the possibility that the malformations observed were not produced by corticosterone hypersecretion as a result of the surgical procedures and amniocentesis.

2. Puncture of fetal membranes via the dilated uterine cervix of intact rats on the 15th day of gestation.

3. Bacteriologic culture of amniotic fluid samples following amniocentesis.

Part A (continued)

4. Amniotic fluid hydrostatic pressure by direct manometry employing a physiological pressure transducer and a needle-tipped catheter introduced into the amniotic sac after passage through the cervical canal.

5. Determination of amniotic fluid volume by dilution of I^{131} labeled serum albumin injected into amniotic cavity following extirpation of the intact fetal membranes and placenta.

Major Findings

A. Amniocentesis

1. Cleft palate and limb deformities were the principal defects induced. Palatal clefts occurred following amniocentesis on the 14th, 15th, or 16th gestational day. On the other hand, limb deformities were seen when the procedure was performed on any day except the 13th. The greatest incidence of both types of malformation occurred after amniocentesis on the 15th day of gestation. Increased adrenal steroid output did not play a role in the incidence of malformations since adrenalectomy prior to amniocentesis did not alter the results.

2. Puncture of fetal membranes through the cervical canal results in the same types of malformations noted above, with a similar frequency. Following amniocentesis by this method, polyhydramnios was observed in some instances. Although this was not a constant result of amniocentesis it is interesting that this "rebound" effect did occur and the explanation for it, at the present time, is just as obscure as the explanation for the origin of the amniotic fluid itself. The incidence of palatal clefts was greater among the experimental fetuses having an excess of amniotic fluid.

3. Blood agar plates and thioglycollate broth showed no growth of microorganisms after seven days incubation at 37°C when inoculated with amniotic fluid on the 17th day of gestation following amniocentesis on the 15th day of gestation. This indicates that infection is not a factor in teratogenesis by this procedure.

B. Amniotic Fluid Hydrostatic Pressure

Uterine motility caused leakage around the catheter such that reproducibility was not obtained, thereby preventing reliable analysis. Application of external methods of pressure measurement are being considered.

Part A (continued)

C. Amniotic Fluid Volume

1. The volume of amniotic fluid in the rat on the 15th day of gestation is 0.320 cc $\pm$ 0.067. These data were obtained from 16 fetuses from 8 different mothers.

2. The weight of amniotic fluid in the rat on the 15th day of gestation was 0.371 grams $\pm$ 0.028.

D. Effect of Thalidomide on the development of the rat fetus

A single fetus exhibited a tumor of the palate which contained bone and a supernumerary pair of incisor teeth. Disturbed morpho-differentiation occurred in the incisor teeth which were not a part of the tumor. Serial sections of jaws from experimental fetuses without such a tumor are being examined for dental or other defects.

Significance to Dental Research:

The identification of environmental factors which affect fetal development and elucidation of the mechanisms by which they act provide a rational basis for reducing the mortality and morbidity of the fetus and newborn.

Proposed Course of Project:

Completion of measurements of normal values for amniotic fluid volume, pressure and density, and determination of the effects of teratogens upon those parameters.

Part B included Yes

PHS-NIH
Individual Project Report
Calendar Year 1962

Part B. Honors, Awards and Publications

Publications other than abstracts from this project:

King, C.T.G. and Kendrick, F. J. Teratogenic Effects of Thalidomide in the Sprague-Dawley Rat. Lancet, in press.

Honors and Awards Relating to this Project:

None

Serial No. NIDR-42
1. Biochemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1962

Part A

Project Title: The Effect of Prenatal Factors on Fetal Development and other Conditions Influencing Oral Diseases

Principal Investigator: Dr. C. T. G. King

Other Investigators: Dr. F. J. Kendrick, Dr. F. J. McClure and Dr. P. N. Baer

Cooperating Units: None

Man Years (calendar year 1962):

Total:	2 1/2
Professional:	1
Other:	1 1/2

Project Description:

Subproject A:

The effect of narcotic and stressor agents on fetal development.

Objectives:

To study the effect of Thalidomide, other narcotics, and stressor agents administered during gestation, on congenital malformations in the Sprague Dawley rat.

Methods Employed:

Pregnant Sprague-Dawley rats were treated with A.C.T.H., corticosterone, 17 hydroxycorticosterone, and additive stress procedures (electroshock at 4°C ambient temperature) at different stages of pregnancy. In addition, animals were force fed Thalidomide at different stages of pregnancy. Another group was force fed glutethimide (Doriden). The animals were killed on the 20th day of pregnancy and the young examined for malformations.

Major Findings:

The Sprague-Dawley strain of rats is extremely resistant to compounds that act as teratogens in other species (rabbits, mice). The only compound that produced congenital malformations was

Thalidomide. This compound is extremely insoluble in water but a fairly smooth suspension was obtained by adding .1% of tween 80. to the mixture. The experiment produced 6.9% grossly malformed fetuses, the malformations ranging from malrotation of the hind limbs to a hamartoma of the palate, accessory incisors, extremely small fetuses (1.5 to 1.9 gms.), and lack of a tail in one case.

Clearing of the specimens revealed other anomalies, the most common being a subcutaneous cartilagenous type mass of tissue from the mid dorsum to the tail of the fetus. Examination of cleared specimens brought the total number of fetal anomalies to 14.6%.

Significance to Dental Research:

This study is designed to elucidate the cause of certain oral diseases, particularly cleft palate and hair lip. By observing the influence of specific teratogenic agents on experimental rats the prenatal factors involved in the development of these diseases may be determined.

Proposed Course of Project:

Since Thalidomide and certain folic acid antagonists are known to produce congenital malformations in humans and laboratory animals, future emphasis will be placed on their mode of action.

Subproject B:

The effect of isoproterenol hydrochloride on salivary gland morphology and caries susceptibility.

Objective:

Removal of the major salivary glands in the rat leads to rampant dental caries induced by a cariogenic diet. Since isoproterenol hydrochloride has produced enlargement of the salivary glands and increased salivation, it seemed possible that an indirect effect on caries susceptibility might occur.

Methods Employed:

Thirteen-day-old Sprague-Dawley rats received 10 mg of isoproterenol hydrochloride daily for 70 ± 5 days. The animals were then placed on a cariogenic diet and at the terminal date the major salivary glands were processed for histological examination and the lower molar teeth scored for caries.

Major Findings:

Isoproterenol caused enlargement of submaxillary and parotid glands but did not affect the sublingual gland. Excessive salivation was evident. The animals receiving the compound had a higher caries score (50%) than the saline injected controls.

Histologically, the thyroid of these animals seems to be in an active state whereas the submaxillary glands showed hypertrophy of their acinar cells which are filled with granules, and atrophy of the cells of the tubules which were mostly cuboidal and devoid of granules. The parotid glands reacted in a similar manner, i.e., a marked hypertrophy and filling of the acinar cells with granules and an atrophy of the tubular cells.

Significance to Dental Research:

This project attempts to evaluate the relationship of salivary secretions to dental caries.

Proposed Course of Project:

Continued study utilizing more refined technics in order to substantiate the preliminary findings.

Subproject C:

The effect of extirpation of salivary and pituitary glands on calculus formation in the rat.

Objectives:

This study attempts to determine the influence on calculus formation of (a) extirpation of the submaxillary glands alone, (b) complete removal of all major salivary glands, (c) hypophysectomy and (d) adrenocorticotrophin and thyroxine hormone replacement therapy after hypophysectomy.

Methods Employed:

The methods employed are reported in detail in Dr. P. N. Baer's Progress Report.

Major Findings:

Extirpation of the submaxillary glands alone did not affect calculus formation, but when all of the major salivary glands were removed calculus formation was inhibited. Hypophysectomy

significantly reduced calculus formation, probably by inducing atrophy of the major salivary gland. This ability was not regained under adrenocorticotrophin therapy but returned to normal under thyroxine therapy which restores the proteolytic activity of the major salivary glands.

Significance to Dental Research:

To evaluate the effect of metabolic disturbance on the production of dental calculus.

Part B included Yes

PHS-NIH
Individual Project Report
Calendar Year 1962

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

King, C. T. G.: Carbon Dioxide and Certain Aspects of Adrenal Function "Man's Dependence on the Earthly Atmosphere." Ed. by K. E. Schaefer, pp. 117-125. Macmillan 1962.

Stanmeyer, W. R., King, C. T. G., Scofield, H., and Colby, R.: The Effect of Prolonged exposure to Carbon Dioxide on Calcification "Man's Dependence on the Earthly Atmosphere." Ed. by K. E. Schaefer, pp. 157-165. Macmillan 1962.

King, C. T. G., Wilk, A. L., and McClure, F. J.: Carbon Dioxide Induced Acidosis in Pregnant Rats and Caries Susceptibility of their Progeny. Proc. Soc. Exp. Biol. & Med., 111, #2, pp. 486-489 (Nov. 1962).

King, C. T. G., and Kendrick, F. J.: Teratogenic Effects of Thalidomide in the Sprague Dawley Rat. The Lancet (Nov. 1962).

Baer, Paul N., King, C. T. G., and White, Carl L.: Studies on Experimental Calculus Formation in the Rat. IV. Effect of Extirpation of the Salivary and Pituitary Glands. J. of Periodontology. In press.

Smith, L. W., Baer, P. N., King, C. T. G., and White, C. L.: Studies on Experimental Calculus Formation in the Rat. III. Calculus Formation as Influenced by High Fat, High Carbohydrate Diets, and Sucrose in The Drinking Water. J. of Periodontology. In press.

Honors and Awards relating to this project:

None

Serial No. NIDR-43
1. Biochemistry
2. Enzyme Chemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1962

Part A

Project Title: I. The Enzymology of Vitamin B_{12} and Its Relation to One-Carbon Metabolism. II. Mechanism of Enzyme Action

Principal Investigator: Dr. Alan Peterkofsky

Other Investigators: None

Cooperating Units: National Heart Institute, Dr. Herbert Weissbach

Man Years (calendar year 1962):

Total:	1-1/2
Professional:	1
Other:	1/2

Project Description:

Objectives:

I. To determine the mechanisms of biochemical transformations and the properties of the enzymes involved. Specific objectives are: a) to characterize enzymatic processes which involve vitamin B_{12}, particularly in the metabolism of one-carbon compounds; b) to study the enzymatic reactions converting vitamin B_{12} to its coenzyme in a cell-free system; c) to examine the role of vitamin B_{12} in the methyl transfer reaction of methionine biosynthesis; and d) to study the enzymology of soluble ribonucleic acid, particularly the role of the minor base components containing methyl groups.

II. To continue a study of the mechanism of action of histidase.

Methods Employed:

Enzymes are purified by standard techniques. Labelled compounds were synthesized and tested in cell-free systems for biosynthesis of coenzyme B_{12} and methionine. RNA is isolated from normal and mutant bacteria and tested for activity in enzymatic reactions. The minor bases are specifically labelled and isolated by chromatographic procedures.

Part A (continued)

Kinetic assays are performed with radioactivity measurements and ultraviolet spectrophotometry, using the Gilford and Cary recording spectrophotometers.

Major Findings:

I. (a) Our previous studies have shown that the conversion of vitamin B_{12} involves the replacement of the cyanide group which is bound to the cobalt by a 5'-deoxyadenosyl group, which originates in adenosine triphosphate. This complex reaction, which also requires an electron donor, apparently takes place in a single step without the formation of any vitamin B_{12}-like intermediates.

A study with the purified enzyme and P^{32}-labelled adenosine triphosphate (ATP) has established that the phosphate groups of the ATP are released as inorganic tripolyphosphate during the biosynthetic reaction. This unique cleavage of ATP coupled to a 5'-deoxyadenosyl transfer reaction has also recently been proposed in the analogous 5'-deoxyadenosyl transfer involved in the methionine activation reaction to form S-adenosylmethionine. The formation of inorganic tripolyphosphate in these two cases offers significant support for the hypothesis that all 5'deoxyadenosyl transfer reactions will be coupled to tripolyphosphate formation.

(b) It is well established that N^5-methyl-tetrahydrofolic acid (CH_3-THF) can transfer its methyl group to homocysteine to form methionine in extracts of pig liver and E. coli. We have now found that s-adenosyl methionine (AMe) is required in both systems, although the methyl group of AMe is not transferred to homocysteine. In the bacterial system, methyl-B_{12} is specifically required for the methyl transfer from CH_3-THF. Methyl-B_{12} transfers its methyl group to homocysteine in a reaction that is not dependent on AMe. Enzyme-bound methyl-B_{12} appears to be an intermediate in the over-all process; AMe seems to participate in the transfer of the methyl group from CH_3-THF to the B_{12} enzyme.

A requirement for methyl B_{12} for methionine biosynthesis in the mammalian system has not been found. However, when the biosynthetic reaction is carried out under aerobic conditions, there is a nonspecific stimulation by cobamides. This appears to be identical with the protective effect of cobamide compounds that has previously been described for the phosphoroclastic reaction.

(c) A project concerned with the structure and biological activity of soluble ribonucleic acid (s-RNA) is being conducted by other members of this laboratory. Advantage has been taken of the recent report in the literature that a certain methionine auxotroph of E. coli can be cultured to produce s-RNA in which the minor base constituents are lacking. We have purified this type of s-RNA and compared its biological activity to normal s-RNA in an attempt to correlate the necessity of minor base constituents with biological activity. Preliminary studies suggest that the s-RNA which lacks methylated bases does not have the same activity as normal s-RNA. Other studies under way indicate that this same methionine auxotroph of E. coli presents a unique way of specifically labelling the minor methylated bases in s-RNA. This technique will be useful in the structural analysis of s-RNA.

II. The mechanism of action of the enzyme histidase is not yet elucidated. In this study, the unusual inhibition of the enzyme by cysteine was reinvestigated, and the inhibition of the enzyme by cysteine and other structural analogs found competitive with histidine, the substrate for the enzyme. Inhibition by different compounds was correlated with their capacity to form metal chelates. These findings support the idea that there is an enzyme-bound metal at the catalytic site for histidase and that the enzymatic reaction proceeds by a chelation mechanism.

Significance to Dental Research:

The fundamental nature of all biological processes at the enzymatic level is required for a full understanding of these systems in health and disease.

Proposed Course of Project:

I. (a) Studies along similar lines will define more closely the mechanism of action of the enzyme for biosynthesis of coenzyme B_{12}.

(b) Attempts will be made to elucidate more clearly the differences in methionine biosynthesis in mammalian and bacterial systems. The existence of enzyme-bound methyl-B_{12} and the role of AMe in this process will be examined.

(c) The structural analysis of s-RNA with major emphasis on the methylated minor bases will be continued. The nature of the biological specificity of s-RNA will be further examined.

Part A (continued)

II. Further purification of the enzyme histidase will be attempted to more definitely establish the presence of enzyme-bound metal.

Part B included Yes

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Individual Project Report
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Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Peterkofsky, A.: The Mechanism of Action of Histidase: Amino-enzyme formation and Partial Reactions. J. Biol. Chem. 237, 787 (1962).

Weissbach, H., Redfield, B., and Peterkofsky, A. Biosynthesis of the B_{12} Coenzyme: Requirements for Release of Cyanide and Spectral Changes. J. Biol. Chem. 237, 3217 (1962).

Weissbach, H., Peterkofsky, A., and Barker, H. A. The Cobamide Coenzymes in Comprehensive Biochemistry, Volume III, Florkin and Stotz, in press.

Peterkofsky, A., and Weissbach, H. Release of Inorganic Tripolyphosphate from ATP during B_{12} Coenzyme Biosynthesis. J. Biol. Chem., in press.

Honors and Awards relating to this project:

None

Serial No. NIDR-44
1. Biochemistry
2. Protein Chemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1962

Part A

Project Title: Studies on the Mechanism by which Endotoxins Induce Non-specific Resistance in Mammals.

Principal Investigators: Dr. E. Schiffmann

Other Investigators: Dr. S. E. Mergenhagen and Dr. G. R. Martin

Cooperating Units: None

Man Years (calendar year 1962):
Total: 1/2
Professional: 1/4
Other: 1/4

Project Description:

Objectives:

Endotoxin from the organism Neisseria meningitidis upon injection into mice, after an initial lag period, increases resistance to an otherwise lethal challenge by an E. coli strain. The endotoxin produces toxic effects; can be lethal in small doses; is pyrogenic, and can prepare an animal for a Shwarzman reaction.

A crude lipid component isolated from an acidic digest of endotoxin also induces resistance to infection in mice, with the exception that there is no lag period before the increase in resistance.

These findings suggested that the lipid is the active material in stimulating resistance and that an enzymatic release of lipid from endotoxin must occur within the host before its resistance increases. The objectives in this work were: to isolate and characterize the lipid, to demonstrate a release of biologically active lipid from endotoxin by the action of various tissue fractions; and, ultimately to clarify processes by which the toxin stimulates host resistance to disease.

Methods Employed:

(1) Toxin Preparation.

Endotoxin was obtained by phenol-water extraction of Group C Neisseria meningitidis cells and separated from nucleic acids to a large extent by repeated ultracentrifugation.

(2) Isolation and Characterization of Lipid

Toxin, refluxed with 80% acetic acid for 2 hours under nitrogen, was extracted with chloroform to remove lipid material. The "crude" lipid was partially purified by repeated ether-acetone precipitations. Thin-layer chromatography was used to examine both "crude" and "purified" lipid, and some qualitative tests were performed to identify the lipid.

(3) Use of Tissue Fractions and Animal Assays

Mouse liver homogenates were prepared in a fortified medium, and various fractions were tested for their ability to release lipid capable of stimulating resistance to infection in mice. In order to assess the effect of lipid upon the reticulo-endothelial system the numbers of bacteria were counted in portions of liver and spleen from mice treated with lipid and subsequently infected. Such numbers were compared with those from mice not treated with lipid.

Major Findings:

(1) Characterization of the Lipid

The partially purified lipid behaved like a phospholipid in its solubility and chromatographic properties. The material contains phosphate; it reacts with ninhydrin; it gives no test for choline, it shows the presence of unsaturation by the iodine test; and it is positive towards 2,4 dinitrophenylhydrazine. Tentatively, the lipid may be said to contain cephalin with some plasmalogen.

(2) Whole Animal Results

When injected into mice the partially purified lipid gave the following effects after the animals were challenged by a coliform organism: no lag phase in increasing resistance was observed, which on the other hand was the case for the endotoxin; liver and spleen from these mice took up increased numbers of E. coli cells compared to these organs from non-lipid treated animals; and the treated mice were not protected against the toxic effects of endotoxin. These findings suggest that the partially purified lipid was not contaminated with endotoxin.

(3) In Vitro Findings

An active lipid could be isolated from an incubation mixture of mouse liver homogenate and endotoxin. Both bioassays and studies with C^{14} endotoxin indicate that a particulate fraction from liver sedimenting between 5000-20000 X G is active in releasing lipid from toxin. This suggests that in vivo the protective effect of endotoxin is only produced after the liver enzymatically releases lipid from the lipopolysaccharide.

Significance to Dental Research:

The endotoxin is derived from oral biota and is known to produce inflammatory reactions. In addition, the demonstrated systemic effect of toxin in inducing resistance may underline the role of certain oral microorganisms in determining host susceptibility to disease.

Proposed Course of Project:

It is planned to characterize more fully the active lipid released by acidic digestion of endotoxin and to compare this with the biologically active lipid released by incubating a liver fraction with endotoxin. It is hoped that some information can be gained on the nature of the linkage between polysaccharide and lipid.

Part B included Yes

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Individual Project Report
Calendar Year 1962

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Mergenhagen, S. E., Martin, G. R., and Schiffmann, E.: Studies on the Endotoxin of the Group C Meningococcus. J. Immunol. In press.

Honors and Awards relating to this project:

None

203

Serial No. NIDR-45
1. Biochemistry
2. Protein Chemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1962

Part A

Project Title: Factors in the Calcification of Mammalian Aortas and Certain Matrices

Principal Investigator: Dr. Elliott Schiffmann

Other Investigators: Dr. George Martin

Cooperating Units: None

Man Years (calendar year 1962):
Total: 3/4
Professional: 1/2
Other: 1/4

Project Description:

Objectives:

Our work has previously shown that mineralization of rat aortas in serum is dependent upon a number of factors. In the serum, a destruction of labile inhibitors is necessary before calcification. In the matrix, the elastin fibers are the most heavily mineralized components. This finding accords with observations on the site of hydroxy-apatite deposition in atheromatous aortas. The objectives in this work are:

1. To gain some insight into the processes involved in pathological calcification.

2. To examine both matrix and medium requirements for normal mineralization.

Methods Employed:

Rat aortas and serum were incubated under a variety of conditions. Ca^{45} contents of tissues were assayed, and sites of calcification in aortas determined by electron microscopy and standard laboratory procedures.

Major Findings:

Previously, histological examination of aortas and treatment of

tissue with elastase implicated the elastin fibers as the mineralizing component. Electron microscopy and micro-radiography have confirmed this. It has also been found as a result of experiments with serum ultrafiltrates that during the aging of serum a greater proportion of dialyzable calcium is produced. This effect, together with that of the destruction of inhibitors of mineralization during aging, could account for the superior calcifying power of aged compared to fresh serum.

In an attempt to clarify the medium requirements for aortic calcification we have examined the mineralization of this tissue in calcifying solutions. No appreciable hydroxyapatite deposition occurred during incubation in the absence of aorta. Also, certain amino acids such as serine appeared to inhibit calcification. This last observation may conceivably relate to regulatory processes for mineralization occurring in vivo.

In an extension of these studies to other tissues we have been able to mineralize in calcifying solutions, elastin slices obtained from ligamentum nuchae of calf. As in the case with aortas no hydroxyapatite deposition was observed in the absence of the elastin. This material, after exposure to strong oxidizing conditions, showed enhanced mineralization, while pretreatment of the slices with amine-blocking reagents depressed calcification appreciably. Thus it appears that calcification of aortas in serum depends on an appropriate concentration of free calcium and on a disappearance of inhibitors sensitive to alkaline phosphatase.

Evidently elastin, a "poorly ordered" protein, is the predominantly mineralizing component in the aorta. It is conceivable that both free amino and hydroxyl groups are involved in primary sites of mineralization on elastin.

Significance to Dental Research:

The processes in the mineralization of hard tissues are poorly understood. A study of the requirements for calcification in a readily mineralizing system such as aortas may shed light on characteristics of normally calcifying systems.

Proposed Course of Project:

It is proposed to study changes occurring in serum prior to aortic mineralization; the characterization of peptide sequences associated with hydroxyapatite on both aortic and bone matrices; and metabolic events associated with in vitro mineralization of

bones. Information on the relation between atheromatous plaque formation and calcification in the aorta may be obtained.

Part B included Yes

PHS-NIH
Individual Project Report
Calendar Year 1962

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Schiffmann, E., and G. R. Martin: In Vitro Calcification in Rat Aorta in Serum. Nature 194, 189 (1962).

Martin, G. R., E. Schiffmann, N. A. Bladen and M. Nylen: Chemical and Morphological Studies on the In Vitro Calcification of Aorta. J. Cell Biology (In press).

Honors and Awards Relating to this Project:

None

NATIONAL INSTITUTE OF DENTAL RESEARCH

Individual Project Reports
Calendar Year 1962

EPIDEMIOLOGY & BIOMETRY BRANCH:

46. A. L. Russell: Epidemiology of Oral Diseases among Alaskan Natives

47. N. W. Littleton: Epidemiology of Oral Diseases among Ethiopians

48. A. L. Russell and E. C. Leatherwood: Epidemiology of Oral Diseases in Vietnam

49. E. C. Leatherwood: Epidemiology of Oral Diseases in Thailand

50. C. J. Donnelly: Epidemiology of Oral Diseases in Colombia, South America

51. A. L. Russell: Epidemiology of Oral Diseases in Lebanon

52. N. W. Littleton: Epidemiology of Oral Diseases in Burma

53. A. L. Russell: The Epidemiology of Dental Caries - Including the Fluoride-Dental Caries Relationship

54. N. W. Littleton: Dental Caries in Children on Long Term Antibiotic Therapy

55. H. R. Englander: Study of Periodontal and Oral Hygiene Status, and Dental Caries Experience of Teen-Agers in Optimum and Fluoride-Deficient Cities

56. C. J. Donnelly: Clinical Trial of a Dentifrice Containing 10% Soluble Phosphate

57. C. L. White: Relation Between Dental Caries and Periodontal Diseases

58. L. F. Mills: A Study of Dental Morphology: An investigation of anatomical factors in orthodontic problems

Annual Report of the Epidemiology and Biometry Branch
National Institute of Dental Research
Summary Statement

Research activities of the Epidemiology and Biometry Branch continued in 1962 to be concerned with obtaining and analyzing data to elucidate the descriptive and determinative epidemiology of oral diseases. Because specific etiologies of most oral diseases are unknown and appear to be complicated and dynamic interactions between numerous factors within the agent-host-environment relationship, investigations have been necessarily broad in scope. Objectives of studies undertaken by the Branch have varied widely within a broad program which included: (a) Nutrition surveys in co-operation with the Interdepartmental Committee on Nutrition for National Defense; (b) studies of the fluoride-dental caries relationship, including a survey of dental fluorosis; (c) clinical trials of other caries inhibitory agents; (d) investigation of the Keyes-Fitzgerald hypothesis of the communicability of dental caries in animals, to determine whether it is applicable to human caries; (e) a survey of occlusal anamolies in a population; and (f) studies of the epidemiological characteristics of periodontal diseases.

Some of these projects, described in the following sections, were initiated during the year; others were continuations of studies begun in previous years.

Nutrition Surveys

Investigators from this Branch continued to collaborate with the Interdepartmental Committee on Nutrition for National Defense in surveys designed to evaluate the nutritional status of selected populations of various foreign countries. Findings from studies in Vietnam, Lebanon and Burma were under analyses. To date field examinations have been completed in fourteen countries or geographical units in such diverse areas as Africa, North and South America, Southeast Asia and the Middle East. These surveys employed a team approach and within this framework dental epidemiologists worked closely with specialists in nutrition, medicine, biochemistry, food technology and agriculture. During the course of these studies oral examinations were done on thousands of persons. Examinees represented diverse ethnic, social and cultural backgrounds, lived under widely different environmental conditions and subsisted on traditional diets of varying quality and composition.

Vast quantities of information to describe the prevalence and severity of oral diseases in groups with divergent characteristics were obtained through participation in these surveys. Findings have been analyzed for individual studies and collectively as components of an overall program. Attention has been directed toward contrasting findings within and between groups in an effort to better understand the relative significance of contributory factors in the complex etiology of oral diseases--particularly dental caries and periodontal diseases.

Results of work during 1962 on this continuing program provided additional information on the occurrence of oral diseases and corroborated and expanded previous concepts. To date results indicate that prevalence of dental caries

varies widely. High prevalence levels, similar to those reported for the continental United States, were seen in the larger villages of Alaska, throughout Trinidad and in most areas surveyed in South America. Conversely, a very low attack rate from dental caries was observed in Ethiopia, an average of less than one tooth per person was affected at all ages up through 40 years. Dental caries was almost as rare in Eskimos of remote Alaskan villages, in South Vietnam, in Palestinian refugees in Lebanon and in Burma. Preliminary analyses have elicited no consistent relations between dietary or nutritional findings and dental caries except a general tendency for low caries prevalence in areas where caloric intake was marginal or where use of refined carbohydrate was limited.

Generally, periodontal diseases constituted a much greater problem than dental caries in most of these populations. Disease levels lower than those commonly observed in groups within the United States were seen only in remote areas of Alaska and in the Jivaros of Ecuador. Gingival disease with relatively little tooth loss from this cause was reported from Ethiopia. Elsewhere the onset of periodontal diseases was early and advanced destruction was common even in young individuals. In adults, prevalence approached 100 per cent and severity was extreme. Despite a favorable dental caries experience, as many Lebanese as United States citizens were edentulous in middle life due to tooth loss from destruction of supportive tissues. Equally high levels of disease were seen in Trinidad and the three countries of Southeast Asia--Vietnam, Thailand and Burma. As yet no consistent patterns of association between nutrition and periodontal diseases have been developed from these data.

Fluorine and Dental Caries

The pioneer study of the practicality of domestic water fluoridation was initiated in Grand Rapids, Michigan, beginning in January, 1945. This study was intended to determine if controlled fluoridation of water would result in the same inhibition of dental caries as seen in persons using water containing natural fluoride. Efficacy of fluoridation was obvious after a few years of observation. The final field examination required by design of this study, a survey of dental fluorosis in children aged 12-14 years, was reported this year. Less fluorosis was found than had been predicted on the basis of observations on children using water with comparable amounts of natural fluoride. Findings from this study support the original estimates of the safety of controlled fluoridation at the recommended level.

After the efficacy of fluoridation became apparent in the Grand Rapids study, a corollary investigation of the fluoride-dental caries relationship was begun to determine in what manner the inhibition of caries was affected. This was designed on a longitudinal basis. Examinations were to be conducted for a period of ten years following fluoridation of water in Prince Georges and Montgomery counties, Maryland, in January, 1952. The tenth year examinations were completed this past March. Repeated observations of the same children have indicated that most of the assumptions accepted from conventional cross sectional field study were valid. Teeth in eruption at the time of fluoridation continued to decay at essentially the same rate as before. Attack rates were less on smooth surfaces of teeth fully calcified, but not in eruption, at the time of fluoridation; but there was little effect on

development of pit-and-fissure lesions in these teeth. Caries of all tooth surfaces was inhibited in teeth still undergoing calcification at the time of fluoridation. Detailed analyses of data from this study are continuing. Results are expected to yield a family of findings ranging from actuarial-type tables which will permit estimation of the impact of fluoridation upon public health dental programs for children, to inferences concerning the sequence and mechanics of development and calcification of teeth.

Another study of the effects of fluoride on dental and oral health is currently in progress. It was designed primarily to investigate the epidemiological characteristics of periodontal diseases and the relation of these diseases to deposits of oral debris and calculus in children using fluoride free and fluoridated waters. Additionally, information will be obtained on the action of fluoride during the formative periods of the teeth.

Other Studies

The Keyes-Fitzgerald hypothesis that dental caries is a specific, infectious and transmissible disease in laboratory animals raised the obvious question of a parallel process in humans. Microorganisms associated with the carious process in animals were shown to be inhibited by adequate concentrations of penicillin. In a pilot study young rheumatic fever patients who had received penicillin daily, prior to eruption of the permanent teeth, were examined. Dental caries experience in these children was importantly less than in public school children of the same community who had not received antibiotics. Some lesions of caries were observed in the study children. Possibly, drug concentrations were inadequate to completely suppress the contributory effects of the oral flora to the carious process. Plans for a field study presently are being formulated to further investigate these findings.

Animal studies also have suggested that, under certain conditions, phosphate may be an effective inhibitor of dental caries. A clinical trial of the effects on dental caries of a phosphate containing dentifrice is in progress in Pennsylvania. The study was designed for a term of two years, and involves a minimum of three clinical and x-ray examinations.

An epidemiological investigation of possible relations between variations in anatomical characteristics and orthodontic problems was initiated. Clinical examinations have been completed on a selected population of young adult males. Efforts have been directed toward development of criteria to describe prevalence and severity of occlusal anomalies in populations and to use these criteria to elucidate relationships between the factors under study.

In addition to direct research activities, a considerable amount of time was spent in consultation on the design and conduct of field studies being undertaken by others, particularly NIDR research grantees. Biometric services also were furnished in about 17 studies involving NIDR investigators from other laboratories.

Serial No. NIDR-46
1. Epidemiology & Biometry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1962

Part A

Project Title: Epidemiology of Oral Diseases among Alaskan Natives

Principal Investigator: Dr. A. L. Russell

Other Investigator: Mr. C. L. White

Cooperating Units: Interdepartmental Committee on Nutrition for National Defense

Man Years (calendar year 1962):
Total: 3/4
Professional: 1/4
Other: 1/2

Project Description:

Objectives:

To determine the prevalence of dental caries, periodontal diseases, and malocclusion among Alaskan natives and to investigate possible relationships of these disorders to certain nutritional and geographic characteristics of this group.

Methods Employed:

Direct examination of members of the Alaskan National Guard. Subsequently, statistical analysis of data obtained to determine relative risks under varying conditions.

Major Findings:

Dental examinations of approximately 700 Eskimo men of the Alaskan National Guard revealed significant information concerning oral health in this population group. Preliminary analysis of data indicated interesting differences when findings from "primitive" groups were compared with those from groups living under more civilized conditions. Dental caries was virtually non-existent in individuals from remote villages. Many of the primitive groups were essentially free of periodontal diseases. Other similarly primitive groups exhibited a uniformly prevalent, severe gingivitis. Contrary to generally accepted theory, this gingivitis demonstrated little tendency toward progression into chronic destructive periodontal diseases even in the absence of personal oral hygiene or

professional dental care. In contrast to these findings in the more "primitive" groups, Eskimos that had lived for some time under relatively "civilized" conditions, demonstrated prevalence and severity of oral diseases quite comparable to those seen in average male population groups within the United States.

Significance to Dental Research:

The spectrum of living conditions within this Eskimo group affords a unique opportunity to study the influence of "civilization" on oral diseases. Findings from the "primitive" groups are relatively uninfluenced by professional dental care. An opportunity is also present for evaluating oral diseases in terms of the various nutritional patterns included within this population sample.

Proposed Course of Study:

The present phase of study has been concluded. This population, however, can be employed in a crucial test of the hypothesis (established in animals) that dental caries is an infective and transmissible disease once the organisms active in human caries can be identified.

Part B included: Yes

PHS-NIH
Individual Project Report
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Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Mann, G. V., Scott, E. M., Hursh, L. M., Heller, Christine A., Youmans, J. B., Consolazio, C. F., Bridgeforth, E. B., Russell, A. L., and Silverman, M. The health and nutritive status of Alaskan Eskimos. A survey of the Interdepartmental Committee on Nutrition for National Defense - 1950., Am. J. Clin. Nutrition, 11:31-76, July 1962.

Russell, A. L. Periodontal disease in well- and malnourished populations. A preliminary report. Arch. Environmental Health, 5:153-7, Aug. 1962.

Honors and Awards relating to this project:

None

Serial No. NIDR-47
1. Epidemiology & Biometry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1962

Part A

Project Title: Epidemiology of Oral Diseases among Ethiopians

Principal Investigator: Dr. N. W. Littleton

Other Investigator: None

Cooperating Units: Interdepartmental Committee on Nutrition for National Defense.

Man Years (calendar year 1962):
Total: 1/2
Professional: 1/4
Other: 1/4

Project Description:

Objectives:

1. To determine the oral health status in a selected sample of Ethiopians; in particular, to determine the prevalence and epidemiological characteristics of dental caries, periodontal diseases, fluorosis, attrition and the general level of oral hygiene.

2. To study the interrelationships between the several dental findings.

3. To relate these findings to medical nutritional and environmental factors in an effort to establish possible associations.

Methods Employed:

Direct examination of an Ethiopian sample with subsequent analysis of the resulting data to determine relative risk under varying conditions.

Major Findings

Dental caries experience in Ethiopians was importantly lower than that commonly reported from similar studies of populations within the United States. This low prevalence of dental caries occurred

in Ethiopians apparently subsisting on a diet traditionally high in unrefined carbohydrates but low in sugar. Information on fluoride levels tended to indicate that the relative freedom from dental caries was not dependent upon fluoride ingestion.

Periodontal diseases were prevalent and severe in the Ethiopian sample. No unique or unusual clinical signs were observed. Severity of periodontal disease was significantly associated with general levels of oral calculus and debris. Nutritional factors appeared to exert only a minor influence on the status of periodontal tissues.

Significance to Dental Research:

This project presented an opportunity to study a population about which little was known concerning oral health status, and one in which the effects of personal oral hygiene and professional dental care were minimal. Findings were used to establish general prevalence levels of certain important oral diseases. Efforts were directed toward determining possible correlations between specific environmental, medical, biochemical and nutritional data and the occurrence of oral diseases.

Proposed Course of Project:

The present phase of study has been concluded. Findings from this study will be intergrated with those from a series of similar studies conducted in other populations in an effort to expand information on epidemiological characteristics of oral diseases.

Part B included: Yes

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Individual Project Report
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Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Submitted for publication in Public Health Reports

Littleton, N. W. Dental findings from a survey in Ethiopia. I. Dental Caries.

Littleton, N. W. Dental findings from a survey in Ethiopia. II. Periodontal Diseases.

Russell, A. L. Periodontal disease in well- and malnourished populations. A preliminary report. Arch. Environmental Health, 5:153-7, Aug. 1962.

Honors and Awards relating to this project:

None

Serial No. NIDR-48
1. Epidemiology & Biometry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1962

Part A

Project Title: Epidemiology of Oral Diseases in Vietnam

Principal Investigators: Dr. A. L. Russell and Dr. E. C. Leatherwood

Other Investigators: None

Cooperating Units: Interdepartmental Committee on Nutrition for National Defense

Man Years (calendar year 1962):
Total: 2
Professional: 3/4
Other: 1-1/4

Project Description:

Objectives:

To determine epidemiological characteristics of oral diseases in a sample population from Vietnam and to relate these findings to certain environmental, medical, biochemical, and nutritional data in an attempt to establish possible associations.

Methods Employed:

Direct examination of population groups followed by statistical analysis of findings to determine relative risk under varying conditions.

Major Findings:

Analysis of these data are still in progress. However, preliminary tabulation indicates that prevalence of dental caries was generally low. While the prevalence of periodontal diseases was widespread in the general population, these conditions were extremely severe in individuals living in certain mountainous areas. These findings occurred in a population subsisting on a soft, rice diet with indications of marginal intakes of thiamine, riboflavin, and Vitamin A.

Significance to Dental Research:

This project presents an opportunity to expand present information

on the epidemiological characteristics of oral diseases. Furthermore, possible associations may be established between the occurrence of oral diseases and environmental, medical, biochemical and nutritional factors.

Proposed Course of Project:

Electronic data processing will be used to explore numerous multiple correlations between oral disease and nutritional and physical findings developed by other members of the ICNND examination team. Data from this study will be collated with data from others in an attempt to improve present knowledge concerning the occurrence and etiology of oral diseases in these population groups.

Part B included: Yes

PHS-NIH
Individual Project Report
Calendar Year 1962

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Russell, A. L. Periodontal disease in well- and malnourished populations. A preliminary report. Arch. Environmental Health, 5: 153-7, Aug. 1962.

Honors and Awards relating to this project:

None

Serial No. NIDR-49
1. Epidemiology & Biometry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1962

Part A

Project Title: Epidemiology of Oral Diseases in Thailand

Principal Investigator: Dr. E. C. Leatherwood

Other Investigators: None

Cooperating Units: Interdepartmental Committee on Nutrition for National Defense and Walter Reed Army Medical Center, Institute of Dental Research

Man Years (calendar year 1962):
Total: 1-1/2
Professional: 1/2
Other: 1

Project Description:

Objectives:

1. To determine the oral health status in military and civilian samples in Thailand; in particular, to determine the prevalence and epidemiological characteristics of dental caries, periodontal diseases, fluorosis, malocclusion, and attrition.

2. To relate these findings to medical, nutritional, and environmental factors in an effort to establish possible associations.

Methods Employed:

Direct examination of military and civilian samples in Thailand with subsequent analysis of data to determine relative risk under varying conditions.

Major Findings:

1. Prevalence of dental caries was extremely low, especially in the younger age groups and even through the age of forty.

2. Prevalence and severity of periodontal disease were quite high and considered to be the major oral health problem.

3. Clinical evidence of fluoride ingestion throughout the country with the prevalence of fluorosis varying from one region to another. At this time, the source of fluoride is in question. Low fluoride levels were reported for water but urinary excretion of fluoride was estimated to be somewhat elevated.

Significance to Dental Research:

This project presented an opportunity to evaluate the prevalence of oral diseases in the population under study. It also offered the opportunity to evaluate the oral health status in terms of certain biochemical, nutritional, and environmental data in an effort to establish possible associations.

Proposed Course of Project:

The principal investigator has left the Branch. However, analysis of data from this study will continue and findings will be incorporated into the overall program of nutritional surveys.

Part B included: Yes

PHS-NIH
Individual Project Report
Calendar Year 1962

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Russell, A. L. Periodontal disease in well- and malnourished populations. A preliminary report. Arch. Environmental Health, 5: 153-7, Aug. 1962.

Honors and Awards relating to this project:

None

Serial No. NIDR-50
1. Epidemiology & Biometry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1962

Part A

Project Title: Epidemiology of Oral Diseases in Colombia, South America

Principal Investigator: Dr. C. J. Donnelly

Other Investigator: None

Cooperating Units: Interdepartmental Committee on Nutrition for National Defense

Man Years (calendar year 1962):
Total: 1-1/4
Professional: 1/2
Other: 3/4

Project Description:

Objectives:

To determine epidemiological characteristics of oral diseases in a sample population from Colombia and to relate these findings to certain environmental, medical, biochemical, and nutritional data in an attempt to establish possible associations.

Methods Employed:

Direct examination of population groups followed by statistical analysis of findings to determine relative risk under varying conditions.

Major Findings:

The prevalence of both caries and periodontal disease was high in the civilian and military groups examined in Colombia. Most water supplies sampled were deficient in fluoride. Caries prevalence was lower where appreciable amounts of fluoride was found in the drinking water. Mild and very mild fluorosis was seen in areas where the fluoride content of the water would in the United States be considered too low to cause any degree of fluorosis. Oral cleanliness correlated well with periodontal disease but no positive correlation has been found between periodontal disease and any of the nutrients examined.

Significance to Dental Research:

This project presents an opportunity to expand present information on the epidemiological characteristics of oral diseases. Furthermore, possible associations may be established between the occurrence of oral diseases and environmental, medical, biochemical, and nutritional factors.

Proposed Course of Project:

The principal investigator has left the Branch. However, data from this study will be incorporated into the overall program of nutritional surveys.

Part B included: Yes

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Individual Project Report
Calendar Year 1962

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Russell, A. L. Periodontal disease in well- and malnourished populations. A preliminary report. Arch. Environmental Health, 5: 153-7, Aug. 1962.

Honors and Awards relating to this project:

None

Serial No. NIDR-51
1. Epidemiology & Biometry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1962

Part A

Project Title: Epidemiology of Oral Diseases in Lebanon

Principal Investigator: Dr. A. L. Russell

Other Investigator: None

Cooperating Units: Interdepartmental Committee on Nutrition for National Defense

Man Years (calendar year 1962):
Total: 1
Professional: 1/4
Other: 3/4

Project Description:

Objectives:

To determine epidemiological characteristics of oral diseases in military personnel, civilians, and Palestinian refugees and to relate these findings to environmental, medical, biochemical, dietary, and nutritional data in an effort to establish relations of possible importance in etiology and control.

Methods Employed:

Direct examinations of population groups followed by statistical analysis of findings to determine relative risk in varying circumstances.

Major Findings:

Analysis of these data is still in progress. Preliminary study indicates that dental caries attack rates were low in all three groups studied. Christian civilian women experienced considerably higher caries attack rates than Moslem women. Dental caries experience of civilian and refugee Moslem men and women did not differ to any extent. Caries attack of Christian refugees were about the same as for Moslem refugees. Periodontal disease was universally prevalent and very severe, particularly in refugees. These differences are associated with differences in custom, diet, and nutrition; some of these associations may have etiological significance.

Significance to Dental Research:

Study of these associations may lead to a better understanding of etiology, or to more effective methods of control, of dental caries and the periodontal diseases.

Proposed Course of Project:

Statistical analysis of the data will be completed and findings correlated with data on environmental condition, biochemical levels of such nutrients as vitamin A, ascorbic acid, thiamine, and riboflavin, and others. Comparisons will then be made with data from similar studies in other countries to determine whether there are factors which tend consistently to modify the pattern or development of disease.

Part B included: Yes

PHS-NIH
Individual Project Report
Calendar Year 1962

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Interdepartmental Committee on Nutrition for National Defense. Republic of Lebanon, Nutrition Survey, Washington, D. C., May 1962.

Honors and Awards relating to this project:

None

Serial No. NIDR-52
1. Epidemiology & Biometry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1962

Part A

Project Title: Epidemiology of Oral Diseases in Burma

Principal Investigator: Dr. N. W. Littleton

Other Investigator: None

Cooperating Units: Interdepartmental Committee on Nutrition for National Defense

Man Years (calendar year 1962):
Total: 1-1/4
Professional: 1/2
Other: 3/4

Project Description:

Objectives:

To determine epidemiological characteristics of oral diseases in the people of Burma, and to relate these findings to environmental, medical, nutritional and biochemical data in an effort to establish possible relations of importance in etiology and control.

Methods Employed:

Direct examinations of population groups followed by statistical analysis of findings to determine relative risk under varying conditions.

Major Findings:

Dental caries prevalence was low in both civilian and military populations. Little clinical evidence of fluoride ingestion was seen. Periodontal diseases were extremely prevalent and severe, particularly in civilians. Preliminary analysis has demonstrated no consistent associations between nutrition and periodontal diseases. Severity of these diseases correlated well with general levels of oral deposits of calculus and debris.

Significance to Dental Research:

This study will investigate whether certain of the factors under

study are important contributor mechanisms in the etiology of oral diseases.

Proposed Course of Project:

Statistical examination of the data and comparison of findings with patterns developed from similar studies in other parts of southeast Asia will continue.

Part B included: Yes

PHS-NIH
Individual Project Report
Calendar Year 1962

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Interdepartmental Committee on Nutrition for National Defense. Union of Burma, Nutrition Survey. Washington, D. C., Dec. 1962.

Honors and Awards relating to this project:

None

Serial No. NIDR-53
1. Epidemiology & Biometry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1962

Part A

Project Title: The Epidemiology of Dental Caries - Including the Fluoride-Dental Caries Relationship

Principal Investigator: Dr. A. L. Russell

Other Investigator: Mr. C. L. White

Cooperating Units: Board of Education, City of Grand Rapids; City-County Health Department, Grand Rapids, Michigan; Michigan Department of Health; Montgomery and Prince Georges Counties, Maryland

Man Years (calendar year 1962):

Total:	2-1/4
Professional:	1/2
Other:	1-3/4

Project Description:

Objectives:

1. To observe the effects of fluoridation of a community water supply on children whose initial exposure to fluoride occurred at different age levels.

2. To determine the epidemiological characteristics of dental caries.

3. To obtain basic information concerning the nature of dental caries and the dental tissues susceptible to attack so that present methods of control may be perfected and, possibly, new methods may be evolved.

Methods Employed:

Direct examination of population groups with subsequent analysis of findings to determine relative risks under various conditions.

Major Findings:

1. Children who use a fluoridated water from birth are protected from dental caries to the same extent and in the same way as children using a natural fluoride water.

2. There was substantial inhibition of caries in teeth which were undergoing calcification at the time of first exposure to water-borne fluoride.

3. There was inhibition of smooth-surface caries, but little of caries in pits and fissures, in teeth which had calcified but which had not yet erupted at the time of fluoridation.

4. Caries inhibition was substantially nil in teeth which were in eruption at the time of fluoridation.

Significance to Dental Research:

Findings from this study are expected to expand present knowledge of the beneficial effects of controlled fluoride ingestion on the dentition. Through detailed analysis, an attempt will be made to secure information on the action of fluoride during the various formative periods of the teeth. Inferences can be drawn on the duration of the inhibitory effects of fluoride on dental caries.

Proposed Course of Project:

The field phase of the Grand Rapids study was ended in December, 1961, with a fluorosis survey of children aged 12-14 years. 1962 is to be the tenth and final year of field examinations in the Maryland study.

Detailed analysis by electronic methods is planned for data from the Maryland study. This was organized on a longitudinal design. A study of year-by-year changes in the status of teeth, surface by surface, is expected to expand present information on the stages of tooth development and the mechanics and duration of the caries-inhibitory effect of fluoride ingestion.

Part B included: Yes

PHS-NIH
Individual Project Report
Calendar Year 1962

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Russell, A. L. Dental caries in first permanent molars after eight years of fluoridation. Arch. Oral Path., 6:50-7 (special supplement), Aug. 1962.

Russell, A. L. The differential diagnosis of fluoride and non-fluoride enamel opacities. Pub. Health Dent., 21:143-6, Winter Issue, 1961-62.

Russell, A. L. Dental fluorosis in Grand Rapids during the seventeenth year of fluoridation. Am. Dent. A. J. 65:608-12, Nov. 1962.

Honors and Awards relating to this project:

None

Serial No. NIDR-54
1. Epidemiology & Biometry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1962

Part A

Project Title: Dental Caries in Children on Long Term Antibiotic Therapy

Principal Investigator: Dr. N. W. Littleton

Other Investigators: Dr. P. H. Keyes and Dr. R. J. Fitzgerald

Cooperating Units: Children's Heart Haven Hospital, Lancaster, Pa.

Man Years (calendar year 1962):
Total: 1
Professional: 1/2
Other: 1/2

Project Description:

Objectives:

Drs. Keyes and Fitzgerald have shown that dental caries in rats and hamsters is an infectious disease, due to a streptococcus specific for each species of animals. This study is the first move in a project to determine whether human dental caries is similarly infectious and transmissible.

Methods Employed:

Examination of a population of children with rheumatic heart disease that had been receiving oral penicillin for some years, beginning at an age prior to the eruption of permanent teeth. Dental caries does not develop in rats or hamsters on an analogous regime.

Major Findings:

Lesions of dental caries were observed in the study children. Possibly drug concentrations were inadequate to completely suppress the contributory effects of the oral flora to the carious process. However, caries attack was consistently less severe in the study children than in children of the same ages not receiving penicillin, living in the same community.

Significance to Dental Research:

If dental caries has a specific etiology it is amenable to specific measures for control.

Proposed Course of Project:

Definitive field study can be carried out once the specific organism or group of organisms responsible for human caries has been identified by microbiological technics. Strong supportive evidence will be adduced if this organism is universally absent in population groups free of caries (such as Nunivak Island Eskimos, Ethiopians, Vietnamese) and universally present in groups in which caries is endemic (as in continental United States). Final demonstration, if the hypotheses survive these tests, may require longitudinal observation and clinical trial with narrow-spectrum antibiotics. Plans are being formulated for further field investigations of these problems.

Part B included: No

Serial No. NIDR-55
1. Epidemiology & Biometry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1962

Part A

Project Title: Study of Periodontal and Oral Hygiene Status, and Dental Caries Experience of Teen-Agers in Optimum and Fluoride-Deficient Cities

Principal Investigator: Dr. H. R. Englander

Other Investigator: Mr. C. L. White

Cooperating Units: Boards of Education, Cities of Aurora and Rockford, Illinois; University of Illinois College of Dentistry, Chicago, Illinois.

Man Years (calendar year 1962):
Total: 1-1/4
Professional: 1
Other: 1/4

Project Description:

Objectives:

1. Primarily to compare the periodontal health of children, aged 13-17 years, who imbibed continuously from birth on optimum concentration of naturally occurring fluoridated water in Aurora, Illinois (1.2 ppm-fluoride) with that of a similar group of native children of a nearby fluoride-deficient city, Rockford, Illinois (0.1 ppm-fluoride).

2. To collect additional information on the epidemiologic characteristics of periodontal disease and to study further the relation of periodontal disease to oral debris and calculus in teen-agers between and within fluoridated and fluoride-deficient cities.

3. To collate these findings with other studies in order to test the usefulness of the oral hygiene index.

4. To determine whether differences in dental caries experience exist among Aurora children who were exposed to fluoridated water during gestation, who were first exposed at birth and who began exposure at 1, 2, 3, 4, 5 and 6 years of age.

Methods Employed:

Clinical examinations of representative groups of the populations, direct interrogation and subsequent analysis of findings according to pertinent variables.

Major Findings:

All data are being tabulated and organized in preparation for analysis and appraisal.

Significance to Dental Research:

A previous survey of adults in Aurora and Rockford has indicated somewhat more favorable periodontal status in Aurorans. This study will determine whether gingivitis is less severe in younger age groups in Aurora and will be the first attempt to compare periodontal health in teen-agers in fluoride and fluoride-deficient cities while factors of oral hygiene are held constant. More knowledge will be gained about the epidemiologic pattern of periodontal disease in teen-agers and additional information on the usefulness of Periodontal and Oral Hygiene Indices will result.

It has been reported in the literature that children who have been exposed to fluorides during gestation receive greater protection from dental caries than those first exposed from birth. The present study will test this hypothesis.

Proposed Course of Study:

1. Studies on the epidemiology of periodontal disease on teen-agers and adults will be conducted in other fluoride and fluoride-deficient cities in order to determine whether patterns of disease similar to those found in Aurora and Rockford exist.

2. Data collected on dental caries experience in Aurora and Rockford will be integrated into other studies.

3. The first study using radiographs to compare dental caries experience of adults in fluoride and fluoride-deficient cities will be completed.

Part B included: Yes

PHS-NIH
Individual Project Report
Calendar Year 1962

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Englander, H. R. and Wallace, D. A. Aurora-Rockford, Illinois, Study III. Effects of Naturally Fluoridated Water on Dental Caries in Adults. Pub. Health Rep. 77: 887-893, Oct. 1962.

2. Englander, H. R., De Palma, R. and Kesel, R. G. Aurora-Rockford, Illinois, Study I. Effects of Water Having Naturally Occurring Fluoride on Dental Health of Young Adults. J.A.D.A. 65: 614-621, Nov. 1962.

Honors and Awards relating to this project:

None

Serial No. NIDR-56
1. Epidemiology & Biometry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1962

Part A

Project Title: Clinical Trial of a Dentifrice Containing 10% Soluble Phosphate

Principal Investigators: Dr. C. J. Donnelly and Dr. F. J. McClure

Other Investigator: Mr. C. L. White

Cooperating Units: Lancaster Cleft Palate Clinic and Milton J. Hershey School

Man Years (calendar year 1962):
Total: 1-1/2
Professional: 3/4
Other: 3/4

Project Description:

Objectives:

To determine if a dentifrice with a soluble phosphate will reduce the incidence of dental caries.

Methods Employed:

A dentifrice containing 10% soluble phosphate is being tested in a double blind study at an institution with 900 boys residing in approximately 50 cottages. The cottages were matched by caries prevalence and equal numbers were randomly assigned to study and control groups. The control dentifrice is similar but without 10% soluble phosphate. A base line of caries prevalence was established by a bite wing radiograph and visual dental examination. Caries incidence will be determined from periodic repetition of the bite-wing and visual examination. Mean incidence of carious teeth and tooth surfaces are to be compared for study and control groups.

Major Findings:

Examinations to establish a base line of dental caries prevalence have been completed. Results indicate that dental caries experience in the study population was high. Random assignments of cottages to test and control groups have been completed. Statistical analysis indicated that past caries experience in these groups were similar.

Significance to Dental Research:

Dietary control of caries is impractical for large groups. Water fluoridation is not readily available to those who are not served by a communal water supply and has been denied to many others by the action of antifluoridationists or by the inaction of local authorities. It would be desirable to have available other effective methods for the mass control of dental caries to supplement fluoridation. Because toothbrushing is a widespread established habit available to all, therapeutic dentifrices offer promise as a mean of inhibiting caries. There is some evidence from animal experimentation that a soluble phosphate is capable of inhibiting caries. Animal experiements further indicate that the action of soluble phosphates may be topical and hence its incorporation in a dentifrice to assure appreciable contact with tooth surface.

Proposed Course of Project:

The study was initiated in 1961 and will be continued for 18-30 months.

Part B included: No

Serial No. NIDR-57
1. Epidemiology & Biometry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1962

Part A

Project Title: Relation Between Dental Caries and Periodontal Diseases

Principal Investigator: Mr. C. L. White

Other Investigator: Dr. A. L. Russell

Cooperating Units: None

Man Years (calendar year 1962):
Total: 1/2
Professional: 1/4
Other: 1/4

Project Description:

Objectives:

To study possible correlations between dental caries, fluoride, and periodontal diseases.

Methods Employed:

Analysis of data obtained from studies on caries and periodontal diseases conducted by the Epidemiology and Biometry Branch.

Major Findings:

1. Preliminary findings indicate that there is no significant difference in the occurrence of periodontal diseases about teeth with or without dental caries.

2. Periodontal tissues are not harmed by use of a fluoride-bearing domestic water.

Significance to Dental Research:

This project expands the program of the Epidemiology and Biometry Branch through statistical investigation of data available from other projects.

Proposed Course of Project:

To continue to expand the study as outlined.

Part B included: Yes

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Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

White, Carl L., and Russell, A. L. Some relations between dental caries experience and active periodontal disease in two thousand adults. N. Y. Dent. J. 32:211-5, June-July 1962.

Honors and Awards relating to this project:

None

Serial No. NIDR-58
1. Epidemiology & Biometry
3. Bethesda, Md.

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Part A

Project Title: A Study of Dental Morphology: An investigation of anatomical factors in orthodontic problems

Principal Investigator: Dr. Loren F. Mills

Other Investigator: Dr. Peter J. Coccaro

Cooperating Units: United States Naval Academy, Annapolis, Md.

Man Years (calendar year 1962):
Total: 1-1/4
Professional: 1
Other: 1/4

Project Description:

Objectives:

To develop criteria for the prevalence and severity of occlusal anomalies in populations; and to apply these criteria in an attempt to elucidate possible relationships between the factors under study.

Methods Employed:

Direct examination of a population of young adult males and measurements of various dental anatomical characteristics considered to be important contributory factors in orthodontic and developmental problems.

Major Findings:

Size of teeth *per se* appeared to have no relationship to the severity of crowding. No relationship could be demonstrated between arch length and crowding or malalignment. An inverse relationship between arch width and crowding was seen.

Significance to Dental Research:

Criteria developed during the course of this study could prove valuable in the investigation of orthodontic problems in populations. These criteria could aid in early preventive orthodontic care.

Proposed Course of Project:

Continue to analyze these data, and to test these findings in a longitudinal study to evaluate their predictive potential.

Part B included: No

NATIONAL INSTITUTE OF DENTAL RESEARCH

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CLINICAL INVESTIGATIONS:

59. R. M. Stephan: Studies on the Etiology and Control of Rampant Dental Caries.

60. H. R. Stanley: Histopathology of the Human Pulp.

61. Herbert Swerdlow: Reaction of the Human Dental Pulp to Cavity Preparations and Filling Materials.

62. P. N. Baer: Studies on Experimental Calculus Formation.

63. P. N. Baer: Treatment of Hyperplastic Gingival Lesions in Patients under Dilantin Therapy.

64. P. N. Baer: Virus and Periodontal Disease

65. Samuel Kakehashi: Comparative Pathology of Periodontal Disease I. Gorilla.

66. H. R. Stanley: Three Dimensional Studies of Periodontal Tissues.

67. E. J. Driscoll: General Anesthesia (Dental) Baseline Data.

68. E. Graykowski: Biochemical and Histochemical Studies in Recurrent Aphthous Stomatitis.

69. H. O. Archard, Jr.: Histopathology of Buccal Mucous Membrane.

70. Jack W. Gamble: Post Surgical Tissue Healing.

71. J. D. Niswander: Oral Aspects of the Results of Consanguineous Marriage in Hiroshima and Nagasaki, Japan.

72. C. J. Witkop, Jr.: Tri-racial Isolates in Eastern United States.

73. C. J. Witkop, Jr.: Genetic Study in Southern Maryland.

74. C. J. Witkop, Jr.: Genetic Studies in a Population Isolate (Brandywine) with Particular Reference to Hemoglobin and Haptoglobin Patterns.

75. C. S. Chung: Sickle-cell Anemia, Other Inherited Hematological Factors and Blood Groups Related to Oral Disease.

CLINICAL INVESTIGATIONS: (continued)

76. C. J. Witkop, Jr. and L. Barros: Hereditary Defects of Enamel and Dentin.

77. K. S. Brown and C. S. Chung: Discrimination and Segregation Analysis of Hereditary Deafness in the Students of the Clarke School for the Deaf.

78. R. O. Wolf: Saliva Study.

79. Herbert L. Cooper: Chromosome and Cell Growth Studies in Normal and Abnormal Subjects.

80. G. Cassady and K. S. Brown: Hereditary Renal Dysfunction with Associated Anomalies - Genetic and Clinical Studies.

81. C. J. Witkop, Jr.: Hereditary Biochemical Defects of Speech, Hearing, and Mastication.

82. J. F. Bosma: Clinical Studies of Oral and Pharyngeal Disabilities Resulting from Local Anomaly, Neurological Impairment, Neoplasm or Surgical Excision.

83. Y. Takagi: Studies on the Pharyngeal Receptor Systems Affecting Fragmental Regulation of Respiration.

84. P. J. Coccaro: Behavior of Points* "A" and "B" in Immediate Denture Patients Utilizing Tantalum Pins.

85. P. J. Coccaro: Longitudinal Study of Soft Tissue Profile in Children with Unilateral Cleft Lip and Palate.

86. W. H. Richmond: Brain Stem Mechanisms in the Integration of Reflex Deglutition.

87. M. J. Baer: Analysis of the Development of Facial and Cranial Skeleton in Mammals as Revealed by Vital Staining.

88. R. C. Grossman: The Sensory Experience of the Mucosal Surfaces of the Oral Cavity.

Annual Report of the Clinical Investigations Branch
National Institute of Dental Research
Summary Statement

Seven years ago, when the Clinical Investigations Branch became a functional unit of the National Institute of Dental Research, the first few years were spent in investigating the common oral and dental diseases which were considered of immediate importance. The studies involved, for the most part, the purely categorical dental diseases, such as caries, periodontitis, and certain prosthetic conditions. Soon thereafter, studies in oral biology, systemic physiology, human genetics and, finally, basic studies in oral and pharyngeal development and function were added. These studies involve basic anatomy, neurology, general pathology, and many other of the medical disciplines which have as their ultimate objectives the understanding and control of oral and dental disease.

For the purpose of this report, the activities of the Branch can best be described in four general categories: (1) Studies involving oral surgery and oral medicine; (2) studies in human genetics; (3) studies in oral and pharyngeal development and function; and (4) dental services provided to the total National Institutes of Health's program.

Oral Surgery and Oral Medicine

Research projects on the oral diseases include those concerned with the teeth, such as caries and erosion, pulpitis and periodontal disease, and those concerned with pathological conditions of the mucous membranes and uderlying oral structures, such as aphthous and herpetic stomatitis, leukoplakia, lichen planus, and desquamative gingivitis.

It is recognized that one cannot dissociate the health of the oral structures from the health of the entire body, and that one cannot dissociate the health of the individual patient from that of the family and the social and genetic community from which he has come. It is also recognized that clinical investigations should include related laboratory studies on physiological and biochemical mechanisms, as well as studies of similar disease conditions in model animal systems.

A brief review of the activities in the broad program of oral surgery and oral medicine follows:

(a) Dental Caries: There is under way a comprehensive clinical and laboratory study of the multiple factors involved in the etiology of rampant dental caries. This study has included the use of advanced instrumentation for making intra-oral observations and measurements of the caries process and the evaluation of predisposing systemic and familial factors in children with this type of caries. Concurrently, multifactorial experiments have been carried out in laboratory animals to determine the effects of the strain of the animal, the cariogenic properties of various diets, and the cariogenic and infectious potentialities of specific types of oral bacteria derived from patients with rampant caries. The fluorescent antibody technique has been used to trace specific microorganisms. Although the study

deals with the etiology of dental caries in all of its complexities, it is hoped that a clarification will evolve of the relative importance of different basic factors concerned in the caries process, with particular reference to the host-parasite relationship as affected by diet.

As more knowledge is gained on the parasitic and cariogenic properties of different microorganisms, particularly in tracing specific organisms using the fluorescent antibody method, a more specific control of potentially cariogenic microorganisms may be developed.

(b) Studies on the Human Dental Pulp: This project is continuing to evaluate the response of the human dental pulp to changes induced by dental drilling procedures and by various restorative and related materials, such as cavity liners. This study has furnished the Profession with some very practical information on operative procedures, particularly in regard to optimal cutting speeds, the proper use of coolants, and modifications in technic necessary for the safe placement of amalgam.

Because of the delayed and reduced inflammatory response of the pulp following high speed cutting technics, the time lag period for the production of reparative dentin has been greatly prolonged. Thus, dentinal tubules remain open and permit the toxic or irritating products of sterilizing agents, cements and silicates to permeate to the pulp tissue and cause further damage. This slow response of the pulp to repair itself is creating a formidable problem in restorative dentistry, especially in the field of full mouth rehabilitation where often the entire coronal dentin is exposed. Experimental drugs designed to reduce sensitivity of teeth and to more effectively seal the dentinal tubules are being sought, as well as drugs and technics to reduce the lag period in which reparative dentin is formed.

(c) Periodontal Disease Investigations: Animal experiments to assess the effect of skim milk and cornstarch on the production of calculus have demonstrated that it is the carbohydrate per se and not the caloric factor which is responsible for the calculus producing effect. Further studies will evaluate the effects of salivary gland and pituitary gland extirpation. Although the exact etiology of calculus formation is not known, these studies are significant in that it is accepted that once calculus has formed it becomes a very important factor in intensifying periodontal disease.

In a more practical approach to certain periodontal problems, findings to date indicate a significant effectiveness of positive pressure appliances for postoperative control of dilantin gingival hyperplasia and recurring gingival hemangiomas.

Other studies on the occurrence of periodontal disease in sub-human primates (the gorilla) have shown a prevalence as high as 76.4%, in a series of 300 skull specimens examined at the Cleveland Museum of Natural History. The most characteristic change was a peculiar and severe vertical type of bone loss, presumably resulting from a confluence of increasing horizontal resorption and a fenestrating type of alveolar bone resorption.

In order to establish a system for correctly evaluating the three dimensional aspects of the periodontal tissues microscopically, another investigation

is seeking to describe a group of so-called "periodontal surface profiles" in the hope of increasing the knowledge and understanding of the periodontium in both normal and diseased conditions. Efforts are also being made to document histologically the essential criteria of periodontosis.

(d) Anesthesia Studies: A collaborative study with the Clinical Center's Anesthesiology Department of general anesthesia in ambulatory dental patients is developing important information concerning the physiological effects of various anesthetic agents and oral surgical procedures. These data provide a continuing record of pulse, blood pressure, arterial O_2 saturation, respiratory phenomena, cortical brain activity and the electrical activity of the heart. The accumulated data of almost five years of study have been and will continue to be used as a baseline of comparison for the new anesthetic drugs which are being introduced for use in oral surgery. All of the agents and combinations of agents commonly used in oral surgery have been used and evaluated in this study with the exception of Fluothane, which drug is next on the schedule for study.

Among the more significant findings from a practical standpoint are:

1) Consistent hypertension in all ambulatory anesthesias, which directly parallels the intensity of the surgical stimulation;

2) preoperative and operative tachycardias in almost 100% of the anesthetics (the preoperative changes in rate being apprehensive in nature whereas the operative changes are due primarily to the pharmacologic action of the intravenous barbiturates and, secondarily, to the surgical stimulation in extremely light anesthetic planes); and

3) depression of arterial oxygen saturation which is a controllable factor related to anesthetic management and drug administration (i.e., avoidance of obstructions and drug overdosage).

Since in some areas there are almost as many general anesthetics administered in dental offices as in the local hospitals and since there are no other such studies being conducted, the basic physiological data from this study should prove important for the specialty of oral surgery and the dental profession in general.

(e) Studies of Soft Tissue Lesions:

1. Recurrent Aphthous Stomatitis: Study of this fairly common and painful disease of unknown etiology is continuing in an attempt to determine possible relationships with bacterial infection, abnormal body metabolism, iron deficiency anemia or hormonal imbalance. A recent finding of significance is the presence of PPLO (pleuro-pneumonia like organisms) not only in the oral tissues of several patients suffering with the disease but in the blood stream as well. This promising direction of study, in collaboration with the Division of Biologics Standards, may provide additional leads as to the role of bacteria in such other conditions as desquamative stomatitis and erosive lichen planus. Therapeutic investigation is also underway on all these stubborn and resistant chronic debilitating diseases.

2. Mucous Membrane Changes Associated with Age and Certain Diseases: It is apparent that human buccal mucosa, although appearing clinically normal, may undergo various changes with the increasing age of the patient. Since the buccal site is frequently biopsied, standards need to be established to eliminate errors in diagnosis due to the age factor. Related studies are providing a description of histopathological changes associated with systemic disease including amyloidosis and multiple myeloma, and an evaluation of mucosal changes following systemic chemotherapy.

3. Postsurgical Tissue Healing: There is some recent evidence in the literature to suggest that, under certain circumstances, removal of impacted and third molars is followed by irreparable damage to the periodontal and soft tissues of adjacent teeth. This damage relates specifically to such factors as type of impaction, presence of pre-existing disease around the second molar, and age and physical condition of the patient. Findings to date promise to provide important information to assist the oral surgeon in making a judgment of postoperative risk whenever he advises the removal of an impaction.

(f) Miscellaneous Studies:

Surgery of any kind on a hemophiliac poses a most serious threat to life. All varieties of drugs, surgical splints and technics have been utilized in an attempt to overcome the hemorrhagic difficulties indigenous to oral surgical procedures on patients with hemophilia. Plasma in large quantities and immeasurable amounts of professional time have to be expended, even after single extractions. Recently Fraction I (fibrinogen fraction of Cohn) which has a concentration of AHG (anti-hemophilic globulin), has been employed in the surgical management of three hemophiliacs. The first two cases involved the extraction of eight and eleven teeth respectively in single operating sessions while in the third case, twenty-five teeth, two impactions and a generalized alveolectomy were performed in a single operation. In no instances were complications noted other than incidental periodic oozing and the infusion of Fraction I. Results, to date, have been most encouraging for a practical approach to oral surgical problems, trauma and elective general surgery in the hemophiliac patient.

Human Genetics

The major new project of the Human Genetics Section undertaken in 1962 was a study of the genetic causes of deafness (in collaboration with the National Institute of Neurological Diseases and Blindness, the Clarke School for the Deaf in Northampton, Massachusetts, and the Bionetics Research Laboratories). Material for analysis consists of genetic and audiometric data on the pupils and alumni of the Clarke School accumulated over a 40-year span. To date, physical, dental and laboratory examinations have been completed on 530 subjects. While representing only a part of the total to be examined, significant physical differences associated with certain forms of deafness are already apparent. Clinical thyroid abnormalities occur in about 10% of the adult alumni and in 6% of the pupils, ages 5 to 17 years. There also is a significant increase in the frequency of non-tasters for PTC among the hereditary deaf, but not the sporadic deaf. Lack of response to the cold caloric vestibular function test is most frequently associated with a history of meningitis.

Data from this and other studies are being processed by an automatic data processing program, SEGRAN, designed and programmed by the statistical activity of the Human Genetics Section. This program represents a major advance in handling genetic data and has widespread application to some of the more complex segregational problems in human genetics.

Studies on the genetically determined constituents of saliva and their relation to normative traits and disease states have overcome some of the major technical difficulties inherent in this area of research. Reproducible electrophoretic patterns of the electrophoresable salivary components have been obtained. One major finding indicates that secretor factor is produced differentially by the various salivary glands, which probably explains failures of previous genetic studies on whole saliva to yield information relative to secretor titer behaving according to genetic theory. A second major advance is the production of an antiserum system in rabbits immunized to human parotid saliva that reacts with some but not all human parotid saliva. This newly discovered genetically determined system appears to be a common polymorphism affecting about 40% of the population. Its significance relative to disease states is being investigated.

Study of hereditary biochemical defects associated with disorders of speech, hearing and mastication have opened up a new concept of speech problems. These studies were conducted with the cooperation of investigators in the National Institute of Arthritis and Metabolic Diseases, who described a specific biochemical defect, histidinemia, that is associated with a speech defect but with no other apparent clinical abnormality. The finding by this Section of a similar situation in Sjogren-Larsson syndrome, where in addition to speech defects there is an associated disorder of mastication, is being intensively investigated because these disorders are theoretically treatable. Hereditary renal dysfunction and deafness appear associated with abnormal serum lipoproteins, but the basic biochemical defect has not been defined.

Cytological methods have now been worked out for the rapid smear technique to assist in the diagnosis of a number of non-neoplastic oral lesions including pemphigus, hereditary benign intraepithelial dyskeratosis, white sponge nevus, Darier's disease, herpes, PPLO infections of the periadenitis mucosa necrotica recurrens type, and a cytological method of following the course of cancer therapy with methotrexate, 5-fluorouricil and 5-fluorodeoxyuridine.

By utilizing the two-dimensional peptide technique (finger printing), no difference could be demonstrated in the peptide patterns of fetal and adult myoglobin similar to that seen in these hemoglobins.

Studies in population genetics and the epidemiology of genetic disease have contributed descriptions of new populations and practical knowledge about blood group distributions in Chileans useful in disaster work. A knowledge of blood group distribution is imperative for the efficient collection and supplying of blood for emergency transfusions.

The first nationwide survey of a representative sample of Chilean civilians and military has determined the frequency distribution for some twenty-six red cell antigens. Utilizing these data, comparisons were made with nutritional

findings, dental caries severity and periodontal disease. No significant correlations could be found between blood phenotype and these states. However, a significant excess of persons with abnormal oral mucosa (scrotal tongue) were blood group O; and in addition a significant excess of persons with abnormal oral mucosa (scrotal tongue) were low or deficient in serum vitamin A. By utilizing a number of genetic markers, including haptoglobins, a new method of calculating genetic distances between populations was devised.

Based on the findings of the dental data from the Interdepartmental Committee on Nutrition for National Defense survey of Chile, a revised formula for calculating the optimal fluoride concentrations allowing for variations in mean maximum temperatures and milk consumption has been proposed:

$$\text{optimum fluoride in ppm} = \frac{.34}{E}$$

$$E = \frac{(-0.038 + 0.0062T)\ W}{.56}$$

where E is the estimated water consumption in ounces per pound of body weight, and W is the average proportion of domestic water in the total daily fluid intake of children 0 to 10 years of age, and T is the mean maximum temperature in degrees Fahrenheit.

The dental treatment requirements for a representative sample of Chilean Army, Navy and Air Force personnel have been determined, giving these Services their first epidemiologically based estimates of these needs.

Multivariate analysis of factors in periodontal disease in Chileans indicates that local factors such as the presence of calculus, oral debris, attrition, gingival recession and age constitute over 90% of the variance in periodontal index scores and that low vitamin and nutritional levels contribute negligible variance. These data do not substantiate the idea that nutritional status contributes significantly to the common forms of periodontal disease.

A new isoantigenic system in baboons that cross-reacts with some but not all other baboon sera and with some but not all human sera has been described. This finding and the finding of two human sera that contained high precipitating serum iso-antigens indicate that these systems may be equally important as the cell antigen systems in determining cross-reactions in blood transfusion problems.

A significant excess of persons with enamel hypoplasia have been found in sibships from ABO and Rh incompatible matings who did not present the overt signs of severe neurological damage (kernicterus). This important finding, coupled with the observation that there is a 10% fetal loss of type A children from A father x O mother matings, forms the basis of a part of the Montgomery County study to ascertain if the surviving 90% of children from such incompatible matings may show a lesser degree of damage such as mental retardation, dyslexia and dysarthria.

Studies of histidine metabolism have definitely identified urocanic acid as a

urinary metabolite in man. Methods for the quantification of urocanic acid and α-formiminoglutamic acid in human urine were described. Both urocanic acid and α-formiminoglutamic acid excretion are increased after oral histidine loading in normal control subjects and in patients with hepatocellular disease. The response to loading in patients with hepatocellular disease is exaggerated.

Experiments with loading albinos with tyrosine, studies of in vitro melanin production and genetic studies indicate that there are two forms of recessive albinism in man. In the more common form, albinos do have tyrosinase activity, and in the rarer form tyrosinase may be lacking or inactive. Pigmentation can be induced in the skin of the former type, but not on a therapeutic level.

The tooth defect in a rare form of vitamin D-resistant rickets was found to resemble that seen in hypophosphatemia, consisting of marked interglobular dentin formation and microfissures traversing the full thickness of dentin from enamel to pulp. This results essentially in a pulp exposure and abscess formation around clinically normal-appearing teeth. The presence of multiple abscesses around clinically normal-appearing teeth may be one of the first signs of this disease.

Mutation rates have been estimated in man for the gonadal dose from a full-mouth series of X-rays. Based on the findings of three independent studies by others that the gonadal dose from a full-mouth X-ray series using modern techniques is about 0.0016r, germinal mutation rates were calculated using the best estimates available for the number of mutable loci in man and the mutation rate per r. If both parents of one million children received a full-mouth X-ray series, we could expect that from 0 to 400 of the children would carry an induced mutation of any type, if these estimates are correct. As some of these mutations would be lethal in utero, even fewer children would show induced defects. This additional genetic load (0 to 400 mutations) was then compared to the additional load expected as the result of treatment before marriage of a single genetic disease (dentinogenesis imperfecta). In one million U. S. children, about 125 would have dentinogenesis imperfecta. By treating all cases prior to marriagable age, 64 additional cases would be added to the next generation as the result of treatment of the parents. It appears that early medical treatment contributes a substantial number of deleterious mutations to subsequent generations and that the potential number of deleterious mutations contributed as the price for X-ray diagnosis is comparatively small.

Chromosomal satellites which have been thought to be associated with certain abnormal body constitutions have been studied in forty normal subjects. Approximately one third of these have shown detectable asymmetry of satellite bodies. A family with an abnormal number one chromosome is being followed for linkage and other studies.

Studies to determine the nature of the mitotic stimulatory effect known to occur when extracts of kidney bean are added to human lymphocyte cultures have made possible a new technique for obtaining a relatively pure suspension of lymphocytes. In addition, a method to assay the mitotic stimulatory properties of phytohemagglutinin has been devised. Morphological variation of the Y chromosome in various strains of hamsters has been described, thereby ending a taxonomic dispute.

In collaboration with the National Institute of Allergy and Infectious Diseases, a particularly important study has been initiated. Simian vacuolating virus (SV-40), when introduced into hamsters directly will, after a period of time, induce tumors. If introduced into hamster cells in culture, these are transformed into malignant cells which, when reimplanted in the hamster, will cause a rapidly developing fatal tumor. Studies on virally transformed cells have shown chromosomal changes induced by the SV-40 virus to be indistinguishable from chromosome changes found in spontaneously occurring malignancies. This virus SV-40 was widespread in monkey kidney cultures, from which polio vaccine was manufactured. Studies on gene, virus and chromosome lead from a fundamental scientific basis into all reaches of human biology. Discoveries and techniques in genetics are useful in virology and infectious disease, metabolic disease and congenital malformations. Accordingly, these several areas are inseparable.

Studies on inbreeding effects of dental characteristics among Japanese children indicate that although there appears to be an increase in periodontal disease with increased inbreeding, the results are inconclusive: Inbred children appear to have more malocclusion than outbred; no consanguinity effect was noted for tooth eruption status, caries, congenitally missing teeth or supernumerary teeth. Abnormalities of tooth number, both supernumerary and congenitally missing, were more prevalent in Japanese than in other reported populations. No clear-cut simple mode of inheritance can be established for supernumerary teeth. Positive associations were found to exist between congenitally missing teeth and hypoplastic teeth and between fused primary teeth and congenitally missing permanent teeth. An association was found between tooth size and a predisposition to "missing" or "extra" teeth. No association in these data could be found between febrile disease in early childhood and enamel defects.

During the past year the Human Genetics Section had thirty scientific articles accepted for publication, including a book on dental genetics, four book chapters, and twenty-five publications in periodical literature. Sixteen of these have been published to date.

Oral Pharyngeal Development

During the past year, the Section on Oral Pharyngeal Development and Function has undergone progressive stabilization and accumulation of personnel, equipment, methods and clientele. Its orientation to the professional variety of its personnel has progressed well and with significant profit in development of concepts. To date, the complement of professionals in this activity includes competency in orthodontics, anthropology, speech, etc. The investigations of each of these persons is concerned with form and function of the mouth, pharynx and larynx.

Activities

The implementation of studies related to feeding, vocalization and speech is now nearly completed by instruments obtained and installed in our working areas, and the construction of a sound-controlled room for speech research.

In cooperation with the Clinical Center Diagnostic Radiology Department and the Photography Department, sound-correlated cineradiographic and cinephotographic studies of the pharynx and mouth performances are under way.

The patterns of skeletal development in the face and adjacent cranium are under study in a variety of mammals. By methods of vital staining with a succession of dyes, the successive sites of bone formation can be distinguished. These sequence patterns of skeletal form development are demonstrated in normal rats and rabbits, and methods of imposing skeletal distortions upon these immature animals are now under exploration. More detailed studies have been initiated to investigate the mechanisms that determine skeletal form. These include observations on the growth of limb bones of chick embryos in organ cultures, and the study of histological changes of bone undergoing mechanical deformation. Clinical abnormalities of facial and cranial skeleton associated with cleft palate are also under study. During the past year we have undertaken an investigation of skeletal deformation in infants and children having marked degree of facial deformity. Anatomical studies of infant cadavers demonstrating cyclops, bifid face, cleft palate and Robin's syndrome are now under way. Infants or children having severe cleft of palate, cebocephaly, glossoptosis or laryngospasm have been admitted for in-hospital study.

Other related activities include the study of progressive distortions of the skeleton associated with disorders of motor function or resulting from multiple dental extractions.

In a collaborative study with the Karolinska Institute, infant respiratory adaptations at birth, and cry and suckle feeding are being analyzed by extensive cinefluorographic and acoustical methods. Upper respiratory participations in natal transition have been described on the basis of cineradiographic and phonetic recording. The physiological and phonetic performance of cry has also been described in normal infants and the directions of abnormality of cry in neurologically impaired infants have been defined. In comparison with these observations in normal infants and previous studies accumulated of normal mature subjects, this investigator group has undertaken physiological studies of speech and feeding actions in a selected clientele of cleft palate infants and children, of the other more severely impaired subjects mentioned above, and of a small number of referred subjects having more common oral or pharyngeal disabilities. The basic methods of these clinical studies are those of cineradiography and acoustical recording. The cineradiographic film is analyzed in a standard manner, with tracing of selected frames. The acoustical record is initially evaluated by ear, and selected portions are portrayed by sonagrams which display frequency and amplitude. Cinephotography, pressure recording and electromyography are employed optionally.

It has become progressively evident to us that the demonstration of motor performance alone is a very incomplete and inaccurate indication of performance. In this oral and pharyngeal region, the motor performance is most intimately related to local sensation, since the motions themselves occasion most of the local sensation, modulate these sensations and are modulated by them. Accordingly, basic studies in cats have been undertaken to decide the

pharyngeal sensations which modify respiration. This investigation has resulted in a preliminary mapping of respiratory-relevent sensations upon the pharynx--the first recognition that differential sensory representation was present in this area. Initial trials are under way also on the stimulation of epipharynx in anomalous infants and children. Preliminary observations were also made of sensation in the hard palate of the dog.

In parallel with these studies of local sensation and motor function, are studies of the central representation of pharyngeal area functions in the brain stem. To implement this project a neurophysiology laboratory has been equipped for observations in experimental animals of neuronal activity in the central nuclei which govern swallow and related functions.

Other studies in the oral pharyngeal field include an extensive exploration of oral sensation in the human, including introduction of histological techniques for the demonstration of neuro-receptors in the oral mucosae and development of instruments for mucosal surface sensory testing. In this connection, a variety of simple plastic forms have been devised which are placed in the mouth and recognized by oral manipulation. These "oral stereognosis forms" have been standardized in a preliminary set of ten for detection of gross deficiencies in the sensory-motor function of neurologically mature, anatomically normal subjects. More subtle and discriminate variations of shape and size of the frames are now in design, manufacture or trial. Findings, to date, have shown a remarkable variety of motion patterns in subjects having anatomical differences, and a discrepancy between functional adequacy and anatomical form and proportion in the mouth and pharynx area. As a result, we are coming progressively to the inference and conclusion that the motor mechanisms of tongue and pharynx are currently or potentially capable of adequate speech and other upper respiratory feeding functions in most of the common variety of discrepancies of form. The critical deficit in many persons having minor or moderate disorders of "portal area" performance is that of local sensory elicitation and modulation of these motor functions, along with such central coordinative disorders as may be developmentally related to these elicitation deficits and distortions.

Activities of the Dental Services Section

In the course of the past calendar year, authorization was given to establish a Section on Dental Services in the Clinical Investigations Branch. This organizational change in no way altered or modified the Dental Department's responsibility for providing the research beneficiaries of the categorical Institutes with an optimum level of dental care. Additionally, however, the Section was given a clearly defined function to assure fulfillment of the research potential of the Dental Clinic, Clinical Center, through an effective utilization of National Institute of Dental Research and other National Institutes of Health patients.

That the above objectives are being met is attested to by the Section's contributions to important programs of research in periodontology, orthodontics, prosthodontics, and operative dentistry. In more specific terms, examples can be cited of a project designed to evaluate the effects of post extraction bone resorption as it relates to: (1) Preoperative conditions such as

degree of periodontal involvement; (2) operative conditions such as immediate versus delayed alveolectomy; and (3) postoperative conditions such as relining and periodic occlusal adjustments of artificial dentures.

In the discharge of its responsibilities to the total NIH research effort, the Section has active collaborations in progress with all the categorical Institutes. The following examples may be cited.

National Cancer Institute. Prosthetic devices, although very often complicated and time consuming to produce, frequently are necessary in the post-surgical management of intra- and extra-oral head and neck cancer. Intra-oral splints for patients who have had large areas of their oral and masticatory apparatus removed, make a dramatic difference in the recovery of these subjects because of emotional, nutritional, hygienic, and other vital factors which lead to earlier physical and mental rehabilitation. In addition, artificial noses, ears and eyes, of such quality as to defy detection by even the critical observer, are produced for National Cancer Institute subjects in an almost routine fashion.

The Section renders further important contributions to the Leukemia Service of the National Cancer Institute in the handling of the myriad of oral problems associated with the disease, such as bleeding, infection, necrosis, etc.

National Heart Institute. Patients with congenital defects, who are to have heart surgery, pose problems of dental management in both the pre- and post-surgical periods. Special considerations also are necessary in the performance of dental operations on patients with hypertension where fear and anxiety present particular problems. In this project, considerable experience and care is required for proper evaluation of anti-hypertensive drugs.

All patients with rheumatic heart disease present particular problems. As an example, in the absence of proper dental care and preparation, even such a simple procedure as oral prophylaxis can precipitate a fatal acute bacterial endocarditis.

National Institute of Arthritis and Metabolic Diseases. In a study of Fraction I of Cohn, a fibrinogen and AHG (anti-hemophilic globulin) concentrate, the Dental Institute's role has been to assess the response of hemophiliac patients to multiple dental extractions. The fraction, being a concentrate, allows the AHG adjustment in plasma without overloading the systemic circulation with plasma fluid. Results, to date, are most encouraging.

Serial No. NIDR-59 (c)
1. Clinical Investigations
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1962

Part A

Project Title: Studies on the Etiology and Control of Rampant Dental Caries.

Principal Investigator: Dr. R. M. Stephan

Other Investigators: None

Cooperating Units: Dental Clinic, Clinical Center, NIH

Man Years (calendar year 1962)
Total: 2 2/3
Professional: 2/3
Other: 2

Project Description:

Objectives:

Rampant dental caries is a very severe form of dental caries in which practically all of the teeth are attacked by decay in a relatively short period of time. It is found chiefly in young children, but may develop in adults who previously had little or no caries experience. Under suitable experimental conditions, comparable forms of rampant caries can be developed in laboratory animals such as rats and hamsters. From a research standpoint rampant caries offers a most favorable opportunity to study the basic factors which activate or control the caries process because the usually prolonged time element in the development of carious lesions is reduced to a minimum and the determination of caries activity can be much more certain than in caries of usual severity. The purpose of this project is to evaluate in clinical studies the many factors which may be important in different cases of rampant caries, and to study the more important of these factors in laboratory and animal experiments.

Methods Employed:

In addition to the usual oral and medical examinations and laboratory tests, a detailed history is taken in regard to caries experience in the family, to diet illnesses and

Part A (continued)

medication during the period of tooth formation, to the eating habits and habits affecting oral hygiene during the period in which caries developed, and to a comparison with these factors in siblings and other patients with minimal caries. Direct stereomicroscopic observations in the oral cavity are made at 6x-40x magnifications with a Zeiss "Otoscope." Intra-oral pH measurements are made with micro-antimony and glass electrodes and with microapplicators containing colorimetric indicators. Measurements of parotid salivary flow rates are made with the "Lashley Cup" apparatus. Samples of plaques and other material from the teeth, periodontal pockets, and mucous membranes are taken with dental explorers, swabs, and specially designed microscalers for quantitative samples which can be weighed on a microbalance. These samples are studied in microculture slides by phase microscopy, in gram stained smears, in selective culture media and in preparations using the indirect fluorescent antibody technique to label specific microorganisms.

Efforts have been concentrated on isolating specific microorganisms from carious lesions and tooth surfaces in humans, determining the occurrence of these organisms in different cases, and testing the possible cariogenic properties of the microorganisms by inoculation in animal experiments. In animal experiments, the mouths of rats and hamsters are inoculated with pure cultures of bacteria isolated from caries to test the potential cariogenic effect of these organisms. Pretreatment of weanling animals with antibiotics is used in some of the experiments to reduce the resident flora before inoculation. The growth or survival of these microorganisms in the mouths of experimental animals is being followed in plaque samples and smear preparations using the fluorescent antibody method for the demonstration of specific microorganisms. Special attention is also being given to an evaluation of the cariogenic properties of the dietary materials consumed by patients with rampant caries and the further testing of the combined effects of dietary materials and inoculation with microorganisms on experimental caries in animals.

Major Findings:

A comprehensive study has been made for the etiological factors in the development of rampant dental caries in over sixty patients. Members of each patient's family and other dental clinic patients with minimal caries have also been studied as controls.

Part A (continued)

The habit of frequent eating between meals of sweets and refined, high carbohydrate foods has been the most common single factor associated with the development of rampant caries.

Other important factors are: (1) adherent microbic plaque formation on the teeth and infection of tooth substance, (2) the kind and amount of secretions in the oral cavity, and (3) tooth formation, particularly the fluoride intake during the formation of the permanent teeth.

In order to control rampant caries, patients and their parents have been taught to keep detailed records of their eating habits, and to record for several weeks the times when any food or sweets are taken into the mouth. Following this baseline record, they have been instructed to control the between meal eating habit and to reduce the consumption of sweets. The results of this program are being checked by periodic dental examinations and dietary records, and the rapid progress of caries appears to have been greatly reduced in many cases; however, some patients or their parents have found it difficult to follow this program.

In addition, the fluoride content of the drinking water has been checked in children during the tooth formative period, and, if lacking, the proper amount of fluoride has been prescribed.

In animal experiments, it has been found that the addition of large amounts of sugar to various diets of bread, dried skim milk powder, and meat has led to a great increase in the amount of caries produced with these diets.

Thus far, experiments, in which laboratory animals have been inoculated with streptococci isolated from human caries, have not yielded consistent results.

Significance to Dental Research:

The dental profession can generally treat caries successfully by operative and restorative means, but most dentists and patients feel overwhelmed when confronted with the need for filling and refilling all of the teeth in children with rampant caries. They have tried to apply various theories and methods for prevention of caries, but many of these are conflicting and have not solved the problem. Thus, many dentists in this geographic area are generally happy to refer patients with rampant

Part A (continued)

caries to NIDR for study. We are now in a position to make a much more comprehensive study of caries etiology than has previously been done, and to study the local oral and the general systemic condition of patients as they may relate to the carious process. More advanced methods for laboratory investigation and animal experimentation are available to evaluate clinical findings.

Proposed Course of Project:

It is planned to complete the analysis of the large amount of data collected on the studies described here, and to continue to develop the studies using the fluorescent antibody method to trace specific microorganisms related to the development of dental caries. It is also planned to set up further cooperative studies with other investigators who wish to study particular areas of bacteriology or biochemistry in these patients with rampant caries.

Part B included Yes

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Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Howell, A., Jr., Stephan, R. M., and Paul, F.: Prevalence of Actinomyces israelii, A. naeslundii, Bacterionema matruchotii, and Candida albicans in selected areas of the oral cavity and saliva. J. Dent. Res. 41:1050-1059, Sept. -Oct. 1962.

Honors and Awards relating to this project: None

Serial No. NIDR-60 (c)
1. Clinical Investigations
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1962

Part A

Project Title: Histopathology of the Human Pulp

Principal Investigator: Dr. H. R. Stanley

Other Investigators: Drs. H. Swerdlow and James Blythe

Cooperating Units: Dental Department, Clinical Center

Man Years (calendar year 1962):

Total:	1 1/4
Professional:	1/2
Other:	3/4

Project Description:

Objectives:

1. To determine the healing capacity of normal and diseased pulps following drilling procedures per se.

2. To determine the irritating qualities of permanent and temporary filling materials compared to zinc oxide and eugenol. This study will include the effects of silicate cements, self-curing acrylics, and pulpdent. The effects of various cavity liners and sterilizing agents will also be considered.

3. To determine the incidence and time lag interval of reparative dentin under various operative procedures.

4. To determine the range of pathologic findings in the pulp resulting from various degrees of carious involvement.

5. To determine the amount of pathology that might be produced by supercooling of teeth by air-water spray technics.

6. To determine the mitotic frequency of the odontoblasts and other less differentiated cells of the pulp and the time sequence of pulpal regeneration.

Part A (continued)

7. To determine the pulpal response when the cavity preparation reaches into secondary dentin.

Methods Employed:

In the filling material, cavity liner, and sterilization studies, patients with intact teeth to be extracted are selected and Class V cavity preparations cut. The teeth are restored and extracted post-operatively to meet the requirements of the study. After fixation in formalin the teeth are decalcified in 5% formic acid, embedded in paraffin, serially sectioned, and stained with hematoxylin and eosin. Masson's trichrome, Wilder's reticulum stain, Feulgen's reaction, periodic acid-Schiff, toluidine blue, and other special stains are also utilized. Every attempt is made to standardize the categories to be compared in respect to age of the patients, tooth size, postoperative extraction interval, and remaining dentin thickness. The pulpal reactions are compared by recording the incidence of the inflammatory response, lesions predominating in leukocytes, and cellular displacement. The intensity of the inflammatory response and the cellular displacement are also recorded using an arbitrary scale of 0-3.

In the study concerned with the range of pathologic findings in carious teeth, the teeth with various degrees of caries but without restorations are selected for study and extracted without cavity preparations.

Major Findings:

1. Speeds of 50,000 rpm and over, with instruments utilizing the belt-driven or turbine principle, have been found to be less traumatic to the human pulp than technics utilizing 6,000 and 20,000 rpm with the conventional, ball-bearing handpiece. The value of coolants became more significant at the higher speeds. In the absence of adequate coolants, intermittent grinding was of no appreciable benefit. Preparation time at the higher speeds was of little consequence provided frictional heat was controlled. The combination of high speed, controlled temperature, and light load was conducive to minimal pulpal pathologic alteration.

2. When the effects on the pulp of manual versus mechanical condensation of amalgam were compared no significant histopathological differences were found. In the high speed technics, the amalgam raised the intensity of the initially mild response to cavity preparation to a level comparable to that obtained with the lower speed technics followed by zinc oxide and

Part A (continued)

eugenol restorations. The mechanical aspects of amalgam condensation apparently play a role in producing an increased pulpal response regardless of the physical properties of the restorative material itself.

3. When temporary fillings were compared, it was found that gutta percha produced the greatest number of responses with leukocytes predominating in 50% of the lesions as compared to only 6.3% in the ZNOE category. When eucalyptus oil was used with the gutta percha a definite decrease in response was noted. Since heat and pressure in the placement of this filling material are minimized by using this oil, it would appear that these factors contribute to the intensity of the pulpal irritation. The pulpal response with zinc phosphate cement was similar to that seen with temporary stopping. The reaction was not as severe as previously reported by others, but still was much greater than the baseline ZNOE category.

4. When teeth were surveyed for quantity of collagen, it was found that anterior teeth possessed more collagen than posterior teeth and that root pulp contained more collagen than coronal pulp tissue. The amount of collagen did not increase in the coronal pulp after 20 years of age. Bundle collagen increased in the root pulp to 39 years. Diffuse collagen in the root decreased between 10 and 49 years. Teeth containing more than 1.0 mm of irregular dentin filling the pulp chamber had more collagen than did similar teeth with no irregular dentin. From these findings it appears that increased collagen is not a direct result of aging but is a reflection of previous irritation or stimulation to the pulp. A tooth which is stimulated to produce irregular dentin also contains more collagen.

Significance to Dental Research:

1. Contradictory findings in the dental literature leave the profession in a quandary concerning the use of certain operative technics and filling materials. Much of this confusion apparently has emanated from the lack of appreciation of various investigators for the response of the pulp to operative trauma *per se*. Pulpal response can vary in incidence and intensity according to the speed and pressure employed in instrumentation, the thickness of remaining dentin, the post-operative extraction period, the type of coolant, and the size of the cutting tool. All these factors must be considered before evaluating accurately the additional irritating properties of permanent and temporary filling materials, cavity liners, and sterilizing agents.

Part A (continued)

2. Since in clinical practice carious teeth are usually treated, some attempt must be made to relate these studies to carious teeth. Before we can determine the pulpal responses of carious teeth to various controlled technics we must determine some code whereby we can predict the quantity of pathology to be found in the carious teeth prior to experimentation. Some carious teeth present little or no pulpal pathology, whereas others present severe changes. This problem is most acute to the dental profession.

Proposed Course of Project:

1. Since we have shown that high speed technics are biologically safe for the human pulp, emphasis will continue on studies related to the additional effects of filling materials, cavity liners and sterilizing agents.

2. Odontoblasts have been considered incapable of mitotic division. Preodontoblastic cells in the cell rich layer are, however, believed capable of dividing into cells which differentiate into odontoblasts as needed. With the accumulation of a number of specimens revealing mitotic divisions approximating predentin, this whole problem of pulpal regeneration will be re-evaluated.

3. With high speed technics, the traumatic effect of cutting tooth structure is minimal enough that the time lag for reparative dentin is greatly prolonged. Consequently virgin tubules opened by operative technics remain open and permit the filtration of toxic products of cement and silicate to reach the pulp tissues. Measure must be found to either decrease the lag period for reparative dentin formation and/or seal these tubules adequately against permeating substances.

Part B included Yes

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Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Swerdlow, H. and Stanley, H. Response of the human dental pulp to amalgam restoration. J. of Oral Surg., Oral Med., and Oral Path. 15:499-508, April 1962.

2. Stanley, H. R. The cells of the dental pulp. J. Oral Surg., Oral Med., and Oral Path. 15:849-858, July 1962.

3. Dubner, R. and Stanley, H. Response of the human dental pulp to temporary filling materials. J. of Oral Surg., Oral Med., and Oral Path. 15:1009-1017, August 1962.

4. Stanley, H. R. and Ranney, R. R. Age changes in the human dental pulp. I. The quantity of collagen. J. of Oral Surg., Oral Med., and Oral Path. 15:1396-1404, November 1962.

5. Stanley, H. R. Changing concepts of the dental pulp based on recent investigations. J. of the District of Columbia Dental Society, 37:6-9, June 1962.

6. Stanley, H. R. Diseases of the dental pulp. Chapter 25 of Tiecke's textbook on Oral Pathology - in manuscript form.

Honors and Awards Relating to this Project:

Recipient of the Gorgas Odontological Society Award for outstanding contributions to dentistry, April 14, 1962.

Selected as a "discussor" on the Research Panel entitled "New Concepts of Restorative Dentistry" held at the 103rd Annual session of the A.D.A., Miami Beach, Florida, October 30, 1962.

Serial No. NIDR-61 (c)
1. Clinical Investigations
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1962

Part A

Project Title: Reaction of the Human Dental Pulp to Cavity Preparations and Filling Materials

Principal Investigator: Dr. Herbert Swerdlow

Other Investigator: Dr. H. R. Stanley

Cooperating Units: Oral Surgery, Department of the Clinical Center

Man Years (calendar year 1962):

Total:	1
Professional:	1/4
Other:	3/4

Project Description:

Objectives:

During our previous studies the evaluation of the traumatic capacity of the various modern cutting procedures has been completed. The placement of the standard fillings and/or cavity liners (base materials) can now be assessed for their effect upon the vital pulp.

Lack of understanding is profound in clinical dentistry as to the accurate evaluation of the cavity liners, base materials, and restorative materials in the maintenance and protection of the dental pulp.

The accumulating of data on the response to cutting procedures puts us in the position to appraise the biological reaction to filling materials.

Methods Employed:

I. Patients selected for study must have non-carious, non-infected, vital teeth to be extracted for periodontal, prosthetic and/or orthodontic reasons.

Part A (continued)

II. Teeth are prepared with specific regard for the following: (1) rpms (2) cutting tool (3) coolant (4) type and area of tooth to be used (5) time intervals until extraction (6) restorative material.

III. Teeth are extracted at varying intervals. After fixation, sectioning and staining, a histologic evaluation is made, relating the clinical experience of the tooth.

Patient Material:

This year 112 teeth in 21 patients were utilized on this study.

Major Findings:

1. If the health of the pulp is to benefit from the minimal pulpal reactions associated with the new cutting methods, some treatment of the freshly cut dentinal tubules not lined by reparative dentine is imperative prior to restoration.

2. The study completed this year on amalgam demonstrates the necessity for developing a cavity liner or base material capable of protecting and/or sealing the exposed dentinal tubules. This is essential to protect the pulp not only from the external stimuli related to chemical and thermal properties of amalgam, but also from stimuli associated with the condensation of amalgam.

3. The amalgam raised the intensity of the initially mild response to cavity preparation achieved with higher rpm techniques to a level comparable to that obtained with lower rpm techniques followed by zinc oxide-eugenol restorations.

4. No significant differences in pulpal responses were found between hand and mechanical condensation of amalgam when the average intensities of the histopathologic characteristics were compared.

Significance to Dental Research:

1. The development and acceptance of a restorative material, cavity liner, or base material must not only be governed by adequate measures of evaluation for physical properties, but also must conform to rigid biological requirements. The maintenance and protection of the dental pulp and supporting structures is a fundamental principle in restorative dentistry.

Part A (continued)

2. The baseline data accumulated provide the ability to evaluate properly any restorative material introduced for dental procedures.

Proposed Course of Project:

Investigations of the following:

1. Rationale for the use on vital teeth of the corticosteroids.

2. Post-extraction cavity preparation for observation of displacement of odontoblasts.

3. Cavity preparation response with incipient carious teeth.

4. Silicate - an anterior restorative material.

5. Full crown preparations vs. the vertical parallel pin preparation.

5a. Cementing procedures.

6. Horizontal pin-lay.

7. Gold foil vs. amalgam condensation.

Part B included Yes

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Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Swerdlow, H. and Stanley, H.R. Response of the Human Pulp to Amalgam Restorations. Oral Surg., Oral Med., and Oral Path. 15:499 April, 1962.

Honor and Awards Relating to this Project: None

Serial No. NIDR-62 (c)
1. Clinical Investigations
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1962

Part A

Project Title: Studies on Experimental Calculus Formation

Principal Investigator: Dr. P. N. Baer

Other Investigators: Dr. J. Plumbo, Dr. L. Smith, C.L. White

Cooperating Units: None

Man Years (calendar year 1962):

Total:	2/3
Professional:	1/3
Other:	1/3

Project Description:

Subproject A: Effect of Skim Milk and Cornstarch in the Diet

Objectives:

We have previously demonstrated the effect of high carbohydrate diets on the deposition of dental calculus in Sprague-Dawley rats. This investigation was undertaken to determine what effects the following factors might have on calculus deposition:

(a) varying inversely the percentages of skim milk and refined carbohydrate (cornstarch) in the diet,

(b) varying the percentage of the refined carbohydrate (cornstarch), but maintaining the skim milk portion of the diet constant,

(c) maintaining a constant caloric content and reducing the carbohydrate content of the diet.

Part A (continued)

Methods Employed:

Sprague-Dawley weanling rats, of both sexes, were used. They were fed one of the following diets: skim milk, varying from 32% to 78%, while the cornstarch was inversely varied from 66% to 20%; a diet in which only the cornstarch portion was decreased from 60-20%; diets which were identical in caloric content, but varied in that lactalbumin was substituted for the carbohydrate portion. The experimental periods were all 30 days.

Major Findings:

1. The amount of calculus formation was unaffected when the skim milk portion of a calculus forming diet was raised to 78% and the cornstarch reduced to 20% of the diet.

2. A highly significant reduction in calculus formation occurred when the skim milk portion of the diet was maintained at the 32% level and the cornstarch reduced to 20%.

3. In diets containing large quantities of carbohydrate (cornstarch), it is the carbohydrate per se and not the caloric effect of the carbohydrate which appears responsible for its calculus producing effect.

Subproject B: Calculus Formation as Influenced by High Fat, High Carbohydrate Diets, and Sucrose in the Drinking Water.

Objectives:

To determine the efficaciousness of a high fat diet as compared with a high carbohydrate diet in producing calculus and to determine whether concentrations of 5% sucrose in the drinking water would act to inhibit calculus formation.

Methods Employed:

Sprague-Dawley weanling rats, both males and females, were used. The animals were maintained either on a high fat (57% crisco) or a high carbohydrate (66% cornstarch) diet, plus either distilled drinking water or a 5% sucrose containing drinking water. They were kept on these regimes for an experimental period of 30 days in the following two ways: 1.) fed and watered ad libitum; and 2.) pair fed and watered.

Part A (continued)

Major Findings:

1. A high fat diet of a greasy consistency is as effective in causing oral calculus in rats as a high carbohydrate diet of a soft powdery consistency.

2. Drinking solutions containing sucrose, when given ad libitum, generally results in a decrease in calculus formation.

3. Pair fed and watered rats do not show a significant decrease in calculus formation even when sucrose is added to their drinking water.

4. Sucrose containing drinking solutions apparently cause a decrease in calculus formation by inducing the animals to consume more fluids.

Subproject C: Effect of Extirpation of the Salivary Glands and Pituitary Gland.

Objectives:

To determine the influence on calculus formation of:

(a) extirpation of the submaxillary glands,

(b) complete removal of all the major salivary glands,

(c) extirpation of the pituitary gland, and

(d) ACTH and thyroxine as replacement therapy after extirpation of the pituitary.

Methods Employed:

Sprague-Dawley weanling rats, of both sexes, were fed calculus forming diets for a period of 30 days. In addition, they were subjected to one of the following four procedures:

1. extirpation of the submaxillary glands,

2. removal of all the major salivary glands,

3. extirpation of the pituitary gland, or

4. after removal of the pituitary, ACTH or thyroxine was given as replacement therapy.

Part A (continued)

Major Findings:

1. calculus formation in Sprague-Dawley rats is completely inhibited when the major salivary glands are removed or their ducts ligated.

2. removal of the submaxillary glands alone has no effect on the formation of dental calculus.

3. hypophysectomy alone results in a reduction in the formation of dental calculus.

4. thyroxine therapy in hypophysectomized rats completely restores the ability of the animals to form dental calculus.

5. more calculus is formed on the molar teeth of hypophysectomized female rats than male rats.

6. ACTH does not significantly change the ability of the hypophysectomized rats to form calculus. No significant difference is found in the calculus scores between the hypophysectomized animals which receive ACTH and those which receive no replacement therapy.

Significance to Dental Research:

Calculus is believed to be an important etiologic factor in periodontal disease; therefore, all knowledge which can be obtained concerning its formation should be of major interest.

Proposed Course of Project:

Further studies on nutritional and hormonal aspects of calculus formation will be pursued.

Part B included Yes

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Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Smith, L.W., Baer, P.N., King, C.T.G., White, C.L. Studies on Experimental Calculus Formation in the Rat. III. Calculus Formation as Influenced by High Fat, High Carbohydrate Diets, and Sucrose in the Drinking Water. Jr. Perio. (in press).

2. Baer, P.N., King, C.T.G., and White, C.L. Studies on Experimental Calculus Formation in the Rat. IV. Effect of Extirpation of the Salivary Glands and Pituitary Gland. Jr. Perio. (in press).

3. Plumbo, J., Baer, P.N., and White, C.L. Studies on Experimental Calculus Formation in the Rat. V. Effect of Skim Milk and Cornstarch in the Diet. Periodontics. (in press).

Honors and Awards Relating to the Project: None

Serial No. NIDR-63 (c)
1. Clinical Investigations
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1962

Part A

Project Title: Treatment of Hyperplastic Gingival Lesions in Patients under Dilantin Therapy

Principal Investigator: Dr. P. N. Baer

Other Investigators: Dr. R.K. Davis, Dr. J.H. Palmer

Cooperating Units: Children's Center, Laurel, Maryland

Man Years (calendar year 1962)

Total:	2/3
Professional:	1/3
Other:	1/3

Project Description:

Objectives:

To determine whether or not a positive pressure appliance would, under controlled conditions, prevent recurrences of hyperplastic gingival lesions in epileptic patients who have to be maintained on dilantin (diphenylhydantoin) sodium therapy.

Methods Employed:

Eighteen epileptic patients of the Children's Center at Laurel, Maryland, with hyperplastic gingival lesions, who were being treated with dilantin, were divided into two equal groups. One group was treated by surgical excision of the hyperplastic gingival tissue, and this was followed by wearing a pressure appliance at night. The control group was treated also by excision of the hyperplastic tissue, but they did not wear any appliance at any time.

Major Findings:

Positive Pressure Appliances proved to be effective in preventing recurrences of surgically excised gingival hyperplastic tissue in epileptic patients undergoing dilantin (diphenylhydantoin) sodium therapy.

Part A (continued)

Significance to Dental Research:

Since almost 50% of patients undergoing diphenylhydantoin therapy have a gingival hyperplasia, these appliances should prove to be helpful in maintaining oral health and esthetics in a large segment of the patients so afflicted.

Proposed Course of Project:

It will be necessary to carry on long term studies to fully evaluate this type of pressure appliance.

Part B included Yes

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Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Davis, R.K., Baer, P.N., and Palmer, J.H. A Preliminary Report on a New Therapy for Dilantin Gingival Hyperplasia. Jr. Perio. (in press)

Honors and Awards Relating to this Project: None

Serial No. NIDR-64 (c)
1. Clinical Investigations
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1962

Part A

Project Title: Virus and Periodontal Disease

Principal Investigator: Dr. Paul N. Baer

Other Investigators: None

Cooperating Units: Dr. Lawrence Kilham, Dartmouth Medical School, Hanover, New Hampshire

Man Years (calendar year 1962):

Total:	2/3
Professional:	1/3
Other:	1/3

Project Description:

Objectives:

To study the effects of various viruses on the jaws, teeth, and periodontal tissues of several small laboratory animals.

Methods Employed:

Hamsters, which were 5 days of age at the beginning of the experimental period, were inoculated intracerebrally with a 1:10 dilution of Rat Virus. Controls were inoculated with a broth. The animals were sacrificed at various intervals of time from 16 days to 150 days of age.

Major Findings:

1. In the younger hamsters, changes were characterized in the region of the 1st molar, by osteoclastic and cementoclastic resorptions and a bony ankylosis. The 2nd molars showed crowns of normal size and shape, but roots that were short and had an abnormal morphology. The tooth bud of the 3rd molar appeared to be smaller in size than usually seen.

Part A (continued)

2. In the older hamsters, the changes were characterized by 3rd molars with small roots and crowns, necrotic pulps with accompanying periapical lesions, apical migration of the epithelial attachment and alveolar bone loss. Also noted was dens in dente, and supernumerary teeth in the third molar and incisor regions.

Significance to Dental Research:

These studies should contribute to our knowledge of the effects which viruses have on the dental structures.

Proposed Course of Project:

To continue our studies on Rat Virus, and other viruses, as they influence the various dental and oral structures.

Part B included Yes

PHS-NIH
Individual Project Report
Calendar Year 1962

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Baer, P.N. and Kilham, L.: Rat Virus and Periodontal Disease. I. The Periodontium in the Mongoloid Hamster, Oral Surg., Oral Med. and Oral Path. 15:756:1962

2. Baer, P.N. and Kilham, L.: Rat Virus and Periodontal Disease. II. Onset and Effect of Age at Time of Inoculation, Oral Surg., Oral Med., and Oral Path. 15:1302:1962

Honors and Awards Relating to this Project: None

Serial No. NIDR-65 (c)
1. Clinical Investigations
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1962

Part A

Project Title: Comparative Pathology of Periodontal Disease
I. Gorilla.

Principal Investigator: Dr. Samuel Kakehashi

Other Investigators: Dr. Paul N. Baer and Mr. Carl L. White

Cooperating Units: None

Man Years (calendar year 1962):
Total: 1/2
Professional: 1/2
Other: None

Project Description:

Objectives:

To study osseous evidence of periodontal disease in sub-human primates of native habitat.

Methods Employed:

A total of 292 plains specie of gorilla were examined for periodontal disease as evidenced by resorptive lesions of the alveolar process. The skulls were divided as to age and sex. The dentition was examined for: degree and type of alveolar bone loss, presence of anomalies, calculus deposition, presence of carious lesions and the degree of occlusal wear.

Major Findings:

1. Osseous periodontal lesions consisting of horizontal resorption, furcation involvements, interproximal craters, fenestrations and buccal vertical defects were observed in 76.4% of the specimens examined.

Part A (continued)

2. The severe vertical type of bone loss appears to be the result of a confluence of increasing horizontal and fenestration types of alveolar bone resorption.

3. Bone loss and tooth wear both increased with age.

Significance to Dental Research:

This study was undertaken to systematically document osseous evidence of periodontal disease in the gorilla.

Proposed Course of Study:

This is the first of a series of comparative studies to be undertaken for the documentation of periodontal disease in the various higher primate species.

Part B included Yes

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Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Kakehashi, Samuel; Baer, Paul N. and White, Carl L.: Comparative Pathology of Periodontal Disease I. Gorilla, Oral Surg., Oral Med. and Oral Path., in press.

Honors and Awards relating to this project: None

Serial No. NIDR-66 (c)
1. Clinical Investigations
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1962

Part A

Project Title: "Three Dimensional Studies of Periodontal Tissues."

Principal Investigator: Dr. H. R. Stanley

Other Investigators: None

Cooperating Units: Pathologic Anatomy Laboratory of National Cancer Institute, National Institutes of Health

Man Years (calendar year 1962)

Total:	1
Professional:	1/4
Other:	3/4

Project Description:

Objectives:

1. To establish more completely the array of morphohistological changes that occur within the buccal and lingual surfaces of the periodontium.

2. To evaluate the concept of the "Cyclic Phenomenon."

3. To evaluate and determine the incidence of the interproximal periodontal "col".

Methods Employed:

Human jaw specimens are obtained at autopsy, photographed, X-rayed, and measured grossly. Gingival crevices are measured on the buccal, lingual, mesial, and distal surfaces of the teeth. Impressions and casts are constructed. Specimens are then fixed in 10% formalin for at least 72 hours and dimensional changes recorded. After decalcification in 5% formic acid, the specimens are trimmed and blocked through the crowns of the teeth to preserve the interproximal tissues. The tissues are processed routinely, embedded in paraffin and cut serially, keeping track of every stroke of the microtome in order to know the exact depth of penetration. Every fifth and sixth section is mounted and every 20th section is stained with eosin and hematoxylin. Specific distances are measured between certain histologic and pathologic landmarks of the periodontium

Part A (continued)

employing a stage micrometer at x35. Also, the following landmarks are plotted on graph paper: the level of the gingival crest, the lowest point of the calculous plaque, the bottom of the epithelial attachment, and the level of the alveolar crest. When the respective points of every 20th section are connected on the graph, a "profile" of a periodontal surface is produced which enables an investigator to see at a glance all the histologic and pathologic variations and relationships that occur within this heterogenous structure.

Major Findings:

Previous studies have shown that essentially the same results are obtained by the measurement of every 20th section as every section.

Statistical relationships have been established between local pathologic factors as well as their influence upon certain measured distances. For instance we have found that the shape of the subgingival calculus significantly influences three distances that extend respectively from the bottom level of the subgingival calculus to the bottom of the epithelial attachment, to the alveolar crest, and to the deepest level of the inflammatory cells in the periodontal membrane. Their average values were greater when approximating scaly subgingival calculus and smaller when approximating nodular subgingival calculus. A hypothesis termed the "Cyclic Phenomenon" has been proposed to describe an interplay of actions occurring between the gradual build-up of scaly and nodular calcular formations and a diminishing tissue tone within the interdental papilla. When the tissue tone of the papilla is reduced by chronic inflammation to a point that it can no longer determine the shape of the calculus, as exemplified by a shift of the calculus mass from a scaly to a nodular form, the calculus then begins influencing the shape of the interdental papilla. The build-up of the nodular calculus masses on approximating tooth surfaces impinges upon the papilla until the tissue above the impinging masses is severed. Beneath a bridge of calculus formed by the approximating nodular masses, a new papilla regenerates which eventually is again subjected to the same sequence of events.

Significance to Dental Research:

This study has vividly demonstrated how certain histological characteristics appearing in one histologic section can encourage very misleading assumptions and interpretations in respect to the total microscopic picture and especially to treatment planning. It has become obvious that before an investigator can draw any

Part A (continued)

conclusions associating certain characteristics of a periodontal lesion with the presence of calculus, bone resorption, the length and migration of the epithelial attachment, or loss of principle fiber attachment and the pathogenesis of the disease, he must be certain that the associations are maintained through a reasonable thickness of tissue since the characteristics change so frequently.

Proposed Course of Project:

1. Having established the minimal number of step-serial sections needed to include all the morphohistologic variations of the periodontium, many more periodontal "profiles" are to be constructed. This appears to be the best mechanism for revealing the three-dimensional aspects of periodontal lesions. Many more profiles in various situations, both normal and diseases, are to be charted to increase our knowledge and understanding of the total concept of the periodontium and neutralize or destroy many of our erroneous, preconceived notions.

2. The profile technic will also permit us to evaluate the "Cyclic Phenomenon" and the "col", recently described by Cohen of England.

3. To establish the histologic criteria for periodontosis, a disease usually considered to be only a clinical entity without specific microscopic changes.

Part B included Yes

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Part B Honors, Awards and Publications

Publications other than abstracts from this project:

1. Stanley, H. R.: A three dimensional morphologic perspective of periodontal tissues. J.D. Res. Supplement I, Jan. 1962.

2. Stanley, H. R.: The value of three dimensional studies of periodontal lesions - Periodontal surface profiles. J. of Periodontology 33:252-260 July 1962.

Honors and Awards Relating to this project: None

Serial No. NIDR-67 (c)
1. Clinical Investigations
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
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Part A

Project Title: General Anesthesia (Dental) Baseline Data

Principal Investigator: Dr. E. J. Driscoll

Other Investigators: Dr. G. Christenson and Dr. C. Hebert

Cooperating Units: Anesthesiology Department, Clinical Center

Man Years (calendar year 1962):
Total: 1 1/4
Professional: 1/4
Other: 1

Project Description:

Objectives:

For over sixty years in the United States, general anesthesia has been the method of choice for patients who need ambulatory dental anesthesia. This method was employed for the greater part of this era almost exclusively by dentists, despite the knowledge that N_2O hypoxic anesthesia was an admittedly non-physiologic technic. Today, many agents, both volatile and intravenous, are available which no longer necessitate hypoxia. However, the physiologic effects of these drugs on the dental patient have not been studied.

There is a vast amount of scientific information on the hospitalized patient undergoing general anesthesia, but the results of the studies are not applicable to ambulatory anesthesia.

1. Dental general anesthesia is different from ordinary hospital anesthesias in several important respects:

   (a) The patients are ambulatory and not usually sedated;

   (b) the operations are performed in as light a plane as possible;

   (c) the operations are performed in or about the airway; and

Part A (continued)

(d) since the patients expect to be incapacitated only for short periods of time because of these major differences, the background of knowledge in general dental anesthesia is incomplete and inaccurate.

2. The accumulated data from this study have been and will continue to be used as a baseline of comparison for the many new anesthetic drugs which have been and are being proposed for use in oral surgery. (These data will also be valuable if and when electronarcosis is ready for evaluation in dental anesthesia.) The newer drugs that we have already evaluated are the intravenous barbiturates, Methitural and Methohexital, and the volatile fluorinated ether, Fluoromar.

Methods Employed:

1. Full mouth extractions were selected rather than random oral surgical procedures, so that the surgical trauma could be standardized. The teeth are extracted in quadrants, and the entire operation is systematized for time of procedure (average of 20 minutes) and traumatic experience. A four channel polygraph is employed, which is electronically timed to deliver two feet of graph per minute. A staff member records all the anesthetic and operative data accurately timed to the quarter minute (15 seconds) and later transcribes this information on to the polygraph record. Thus, we have an accurately timed chronological narrative on the graph for the evaluation of time-related surgical and anesthetic events, such as the effect of surgical stimulation on pulse and blood pressure.

   The following agents have been evaluated under these controlled conditions:

   (a) Pentothal, Methitural and Methohexital;

   (b) the parasympatholytic antisialogogues, Atropine, Scopolamine, and Hexocyclium;

   (c) various proportions of nitrous oxide oxygen employed as adjuvants (75-25), (80-20), (85-15), these being the usual proportions employed by oral surgeons.

   (d) tri-fluoroethyl-vinyl ether (Fluoromar)--a new halogenated inhalation ether anesthetic.

2. Physiologic data being recorded are pulse, blood pressure, arterial O_2 saturation, respiratory phenomena, cortical brain activity (EEG) and the electrical activity of the heart (EKG).

Part A (continued)

Likewise recorded are such important technical data as amount of drug used in units of time, length of operation, duration of sleep, mental alertness on awakening, age, race, sex, emotional status in relation to the conduct of anesthesia, and post-anesthetic sequelae (headache, nausea and vomiting, depression and hiccoughs).

Patient Material:

Another 150 detailed studies on 50 adult patients were added this calendar year, making an over-all total of 750 operations performed over the last four years.

Major Findings:

1. Sufficient data are now available to determine objectively from brain wave activity the precise anesthetic levels at which various procedures are being carried out with these agents. The addition of N_2O and O_2 does not alter the EEG patterns although the agent Fluoromar does. Short periods of obstruction and hypoxia do not, in our experience, alter the EEG patterns as some investigators have indicated in the literature.

2. The EEG is making it possible for us to state with certainty the precise anesthetic plane which is present at specific intervals when other physiologic measurements such as pulse and pressure are made and studied in relation to trauma.

3. A slight pharmacologic difference has been found in the behavior of Atropine and Tral (Hexocyclium) when employed as an antisialogogue. Both of the agents are given before the barbiturate is administered. Atropine causes an acceleration of the pulse almost immediately, whereas there is no increase in pulse rate when Tral is used until the barbiturate is added.

4. Hexocyclium was found to be a far superior drying agent than Atropine. Another practical feature about Hexocyclium as compared to Atropine is that its action is of short duration; i.e., it clinically reduces secretions very effectively for 20-30 minutes. Thus, in the postoperative period when the patient becomes oriented, there is no annoying problem of a dry mouth.

5. The significant blood pressure elevation and pulse acceleration continues to be our most important finding from a practical standpoint. It was found that the blood pressure and pulse

Part A (continued)

elevation with Methohexital was slightly greater than that we reported with Pentothal and Neraval. Although the values are greater for both pulse and blood pressure, the pattern of rise is identical with the two previously described drugs; i.e., the pulse peaks in one to two minutes, whereas the blood pressure peaks in six minutes indicating a slightly different mechanism in operation in these closely allied physiologic measurements. It was hoped that Fluoromar used along with the barbiturate Methohexital would prevent the rises in pressure and pulse. So far this has not been confirmed, although we have not as yet studied the drug sufficiently.

(a) Alarming tachycardias are being recorded and their onset parallels the first EEG changes which occur as soon as the drug Methohexital passes through the blood brain barrier.

(b) The previously reported hypertension was principally of the systolic variety. We are now ready to report an almost as significant elevation in the diastolic system. The diastolic rise is probably related to the inability of the vascular system to obtain any measure of relaxation because of the rapidly beating heart.

(c) We have enough data to say with certainty that the operative hypertension is related to surgical trauma, and its peak parallels that of the intensity and duration of the stimulation.

(d) The pulse activity gives strong evidence of having a drug (barbiturate) relationship.

Significance to Dental Research:

1. The search is constantly being made in dentistry for safer general anesthetic agents. However, without baseline data on physiologic responses, this search has no foundation. Methohexital is a good example of the new drugs which have been recently introduced, and our studies on this drug demonstrate the value of our previously accumulated data on intravenous barbiturates as far as baselines are concerned. With our previously acquired baselines on Pentothal and Methitural, we are in a good position to evaluate the other new anesthetic drugs which are being introduced for use in ambulatory oral surgery such as Fluoromar and Fluothane.

2. The possibility also exists for improvement and refinement of present methods of anesthesia as well as laying a sound

Part A (continued)

foundation for further anesthesiology research. An example of refinement in methods is indicated in our studies of N_2O and O_2 supplementation and Demerol premedication, both of which help to control adverse rises in blood pressure. Pulse control has so far eluded our therapeutic and technical investigations; however, as we had anticipated, proper premedication does control the pulse and also the pressure to a degree.

Proposed Course of Project:

1. The study will continue on both good risk (ASOS class I) and medium risk (ASOS class II) outpatients. These medium risk patients have nothing wrong with their cardiovascular or respiratory systems which would make them unsuitable candidates for general anesthesia. Additional baseline and comparative data will be accumulated, studied, and evaluated.

2. The studies of Methohexital and the usual supplements will continue. An additional agnet, Fluothane (which is described as causing hypotension and bradycardia), is being considered for study as a possible antagonist to the forces inducing hypertension and tachycardia. Fluoromar was selected for the same reason, but it has poor patient acceptance so far due to its odor, its apparent relationship to vomiting (about 20% of the patients vomited as compared to an approximate 1% incidence in the case of the barbiturates) and its cost.

3. Position (supine versus upright) in the operating chair will also be investigated, as possibly a determinant of adverse pulse change.

4. The effects of vagolytic agents will be further recorded and observed. The earpiece oximeter has been found to be very effective in studies of arterial oxygen saturation.

5. Studies on continuously recorded respiratory data will be further pursued. The recordings are essential to the proper interpretation of arterial oxygen values.

6. Field studies on pulse and blood pressure will be carried out in several oral surgeons' private offices at their invitation. This is believed to be essential to authenticate our findings in different clinical environments.

Part B included Yes

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Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Driscoll, E. J., Christenson, G. R. and White, C. L.: General Anesthesia on Ambulatory Dental Patients--A Resume of a Four Year Study. Journal of Oral Surgery, Anesthesia, and Hospital Dental Service. (In press).

Honors and Awards relating to this project:

Chairman, Committee on Anesthesia, American Society of Oral Surgeons.

Liaison member of the American Society of Oral Surgeons to the American Society of Dental Anesthesiologists.

Serial No. NIDR-68 (c)
1. Clinical Investigations
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1962

Part A

Project Title: "Biochemical and Histochemical Studies in Recurrent Aphthous Stomatitis"

Principal Investigator: Dr. E. Graykowski

Other Investigators: Dr. E. Driscoll

Cooperating Units: Dental Clinic, Clinical Center

Man Years (calendar year 1962)
Total: 2
Professional: 1
Other: 1

Project Description:

Objectives:

The objective is to determine if a relationship exists between the presence of recurrent aphthous oral lesions and the iron metabolism of individuals in which they occur. There are many types of mucosal changes associated with iron deficiency anemia such as cheilosis, glossitis, atrophy of the lingual papillae and various types of nonspecific stomatitis. Could iron deficiency at the cellular or hematological level be associated with aphthous stomatitis? This is an interesting speculation worthy of investigation, since there is some clinical evidence that aphthous ulcers and deficiencies in serum iron (certain anemias) are sometimes correlated.

Continued attempts will be made to establish the role of the oral microbiota in the etiology of the disease.

Methods Employed:

1. The following tests are performed: red blood cell count, white blood cell count and differential, wintrobe indices, sedimentation rate, complement fixation, urinalysis, serum iron, total serum proteins and albumin-globulin ratio.

Part A (continued)

2. The oral mucosa of all patients is biopsied, part of the tissue is sent to the histopathology laboratory and a part is prepared for viral studies. When vesicles are present in the lesion the fluid is aspirated for viral studies.

Patient Material:

Patients are selected from those on record from the previous stomatitis studies and those being referred at the present time to the Clinical Center Dental Clinic. About fifty patients comprise the study group.

Major Findings:

During the course of repeated cultures from the oral mucosa lesions in a case of aphthous stomatitis, periadenitis type, an unusual organism was discovered whose morphologic properties appear to be consistent with the morphology of the PPLO (pleuropneumonia-like organism) group of bacteria. Identical, intracytoplasmic organisms were found in the epithelial cells in tissue sections from these lesions.

Significance to Dental Research:

In view of the fact that the cause of aphthous stomatitis is not known, the consistent finding of this unusual organism in large numbers and in pure culture from numerous lesions in this disease is significant. Further investigation into the relationship of this organism to the pathogenesis of this oral disease is warranted.

Proposed Course of Project:

Serological tests will be utilized to establish that this organism is the causative agent, and the methods of isolation and verification will be applied to the remainder of the study group.

Part B included No

Serial No. NIDR-69 (c)
1. Clinical Investigations
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1962

Part A

Project Title: Histopathology of Buccal Mucous Membrane

Principal Investigator: Dr. H. O. Archard, Jr.

Other Investigators: Dr. H. R. Stanley

Cooperating Units: National Cancer Institute, Medicine Branch, Dr. Paul Carbone
Armed Forces Institute of Pathology, Dental and Oral Division, Capt. L. Hansen, Maj. J. Cornyn

Man Years (calendar year 1962):

Total:	3/4
Professional:	1/2
Other:	1/4

Project Description:

Objectives: To determine:

1. The effect of aging, chemotherapy, and systemic disease processes on the histologic appearance of buccal mucous membrane.

2. The relative incidence and histologic characteristics of disease states of the oral mucosa.

3. The frequency of amyloid deposition in multiple myeloma patients and application of fluorescent stain procedures to this problem.

Methods Employed:

1. Specimens of buccal mucous membrane collected at autopsy and preserved in 10% formalin are studied by routine histologic procedures for evidence of age changes, the effect of chemotherapy, and evidence of systemic disease processes.

2. In conjunction with Captain L. S. Hansen and Major J. Cornyn of the Dental and Oral Division, Armed Forces Institute of Pathology, a statistical analysis of all diseases involving the oral mucosa has been undertaken. A syllabus of diagnostic

Part A (continued)

nomenclature is being prepared by listing the various diagnoses of the mucosal lesions and the specific site in which they are located. Relative frequency of the disease entity is determined from the number of cases for each particular diagnosis.

3. Buccal mucosa specimens from patients with multiple myeloma obtained in vivo and at autopsy, are collected and preserved in 10% formalin. The specimens are examined for amyloid by means of crystal violet staining and by examination with fluorescent dyes and ultraviolet microscopy. In autopsy cases of multiple myeloma, sections of heart, kidney, bowel, as well as buccal mucosa are similarly studied.

Major Findings:

1. Work is still in process for evaluation of changes occurring in buccal mucosa as a result of aging, chemotherapy, and systemic disease.

2. A listing of diagnostic terminology for 14,709 cases having lesions of the lip has been prepared along with the relative incidence of these lesions. This listing serves as a guide to coding of disease entities of this region by the A.F.I.P. staff.

3. The methods described above are still in progress. However, a preliminary report has been prepared on the occurrence of amyloid in 32 autopsy and 17 surgical cases of multiple myeloma.

Significance to Dental Research:

1. Considerable confusion exists at present concerning what constitutes histologically normal and diseased buccal mucous membrane. It is expected that the normal range of histologic variation will be determined for human buccal mucosa. Incipient changes in buccal mucosa, prior to the development of any one of its several recognized disease entities (lichen planus, leukoplakia, hyperkeratosis, and carcinoma), will in the future be better recognized.

2. The frequency and relative occurrence of labial lesions has shown a definite relationship existing between precancerous lesion of the lip and frank carcinoma as it is seen in the A.F.I.P. cases. This relationship is in variance with some schools of thought, and indicates the need for more standardized histologic criteria for the diagnosis of the precancerous lesions.

Part A (continued)

3. It has been determined that the oral biopsy is still just as efficaceous a site for demonstrating amyloid as the rectal biopsy, despite reports to the contrary. However, more material needs to be studied and follow-up of the surgical cases is indicated. Further evaluation of standard and newer staining technics for demonstrating amyloid will be done in order to better characterize this material.

Proposed Course of Project:

Further collection of autopsy and surgical material is needed in the study of changes in buccal mucous membrane histopathology. Critical analysis of this material should then be carried out. Continuation of the methods previously employed in studying amyloidosis in multiple myeloma should be done in order to obtain some relative significance of the occurrence of this material in oral mucosa. Finally, completion of the analysis of oral mucosal lesions is advisable to obtain data for the whole mouth rather than just one restricted area.

Part B included No

Serial No. NIDR-70 (c)
1. Clinical Investigations
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1962

Part A

Project Title: Post Surgical Tissue Healing

Principal Investigator: Dr. Jack W. Gamble

Other Investigator: Dr. Samuel Kakehashi

Cooperating Units: Dental Department, Clinical Center

Man Years (calendar year 1962)
Total: 1 1/2
Professional: 1 1/2
Other: None

Project Description:

Objectives:

A distinct possibility exists for irreparable damage to the periodontal tissues buccal and distal to the mandibular and maxillary permanent second molars during surgical removal of impacted third molars. Accordingly, there is need for a more thorough understanding of the degree of such damage to periodontal tissues. It is feasible to believe that a great amount of information could be gained through a study of tissue healing around maxillary and mandibular second molars as related to:

1. The particular type of impaction involved; i.e. mesio-angular, disto-angular, vertical, or horizontal.

2. The roentgenographic evidence of the presence or absence of a follicle around the crown of the impacted third molar.

3. The presence or absence of pre-existing periodontal disease around the second molar involved.

4. The patient's age at the time of removal of the impacted third molar.

5. The stage of root development of the impacted molar at the time of removal.

Part A (continued)

Methods Employed:

1. Each patient is screened by the oral surgeon. If the patient is accepted for study, the following procedures are performed: (a) History; (b) Laboratory studies including CBC, Hbg, HcT, platelets and differential count; (c) Physical examination; and (d) Panorex roentgenogram.

2. The periodontist evaluates the periodontium of the teeth in the proposed surgical area. This involves the measuring and the recording of all periodontal pockets which are present prior to surgery.

3. Prior to suturing of the soft tissues following the removal of the impacted third molar the height of the bone distal to the second molar is measured and recorded.

4. The periodontist measures and records the periodontal pockets around the second molar at two and six months after surgery.

Patient Material:

Healthy males and females seventeen years of age and older who have impacted third molars are being included in the study.

Major Findings:

To date, twenty-five patients with forty-one impactions have been followed for two months post surgery. Measurements thus far have shown no increase or decrease in depth of pre-existing periodontal pockets buccal or distal to the second molar.

Significance to Dental Research:

Documentation for or against irreparable damage to the periodontal tissues around the second molar secondary to the removal of impacted third molars will aid in determining whether or not non-symptomatic impacted teeth should be removed.

Proposed Course of Project:

Continuation of the project as outlined until two hundred impacted third molars have been removed and post surgical evaluations performed.

Part B included No

Serial No. NIDR-71 (C)
1. Clinical Investigations
2. Human Genetics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1962

Part A

Project Title: Oral Aspects of the Results of Consanguineous Marriage in Hiroshima and Nagasaki, Japan

Principal Investigator: Dr. J. D. Niswander

Other Investigators: Dr. C. J. Witkop, Jr.

Cooperating Units: National Academy of Sciences, Washington, D. C.; University of Michigan, Department of Human Genetics

Man Years (calendar year 1962):

Total:	1
Professional:	1
Other:	None

Project Description:

Objectives:

1. In connection with the genetic program of the Atomic Bomb Casualty Commission in Hiroshima and Nagasaki, Japan, the details of which have been presented by Neel and Schull (Neel, J. V., and Schull, W. J.: The Effect of Exposure to the Atomic Bombs on Pregnancy Termination in Hiroshima and Nagasaki. National Academy of Sciences, #461, Washington, 1956), the outcome of pregnancy was determined in 5,033 instances in which the parents reported consanguinity of some degree. The products of these pregnancies ranged in age between five and ten years at the time they were re-contacted in the present study. In essence, this study involved an attempt to obtain a detailed follow-up study on each of these 5,033 pregnancy terminations, plus a suitable control material.

2. Of the 5,033 pregnancy terminations, a number were known to have resulted in stillbirths or deaths during the first nine months of life. Starting from the records of the Atomic Bomb Casualty Commission, a trained staff of "field workers" reestablished contacts with all of the remaining children (i.e., registered in the Genetics Program and not known to

Part A (continued)

have died during the period of time covered by this program). The result was a roster of living children, a roster of now-deceased children, and a roster of children who have moved from the two study cities. An effort was made to establish cause of death for all deceased children. The background of the children who moved from the cities will be analyzed in a search for biasing factors. The fact of death and, possibly, the cause of death will be analyzed in relation to parental consanguinity.

3. A pilot study carried out in 1956 under the direction of W. J. Schull has already revealed that the death rate among the children of consanguineous marriages is approximately twice the control death rate (cf. Appendix). There is, then, already considerable assurance of significant results from this inquiry.

4. In general, the aim of the dental portion of this study is to provide an assessment of normal variability and disease in and around the oral cavity. This information in conjunction with the other findings provides an overall measure of child health which can be related both "in toto" and by specific components to parental inbreeding.

   In addition to inbreeding effects, these data provide a number of opportunities for comparative racial studies of oral disease and variation. Also a number of investigations of the inter-relationships of oral traits with other variables (e.g., general physical development, socio economic status, medical history, etc.) are being undertaken.

Methods Employed:

Using ABCC facilities in Nagasaki and Hiroshima, a team consisting of specialists in such fields as pediatrics, hematology, dentistry, and anthropology began examinations of offspring of consanguineous marriages and appropriate control groups in September, 1958. Examinations were completed in July, 1960. The following examinations were performed:

1. Physical examination: This consisted of a standard physical examination, with emphasis on characteristics which can be graded in an objective manner lending themselves to statistical analysis.

   a. Presence of gross physical defect; i.e., abnormalities of skin, musculature, skeleton, etc.

Part A (continued)

b. Defects of the auditory mechanism, with special reference to loss of hearing.

c. Defects of the eye, including loss of visual acuity.

d. Cardiac abnormality (cardiomegaly, disorders of rhythm, murmurs, etc.).

e. Pulmonary abnormality.

f. Apparent abnormality of abdominal viscera.

g. Abnormality of external genitalia.

h. Bone age (single wrist film).

j. Determination of leucocyte count and hemoglobin level.

k. Standard urinalysis.

2. The dental examination included pathology of the soft tissues, pathology of the teeth and index of malocclusion, index of tooth loss, developmental failures of oral structures, DMF and def rates and the association of these conditions with and without generalized hereditary disease.

3. Anthropometric examination: This included such standard items as weight, sitting and standing height, span, measurements of head and face, subcutaneous fat index, etc.

4. Psychometrics: Each child was tested at time of clinic visit by means of a portion of the WISC test (maze and trail). In addition, the complete Japanese standardized WISC was administered by the Hiroshima University Department of Psychology.

5. School performance: Access was obtained to school records for each child and selected information abstracted.

All results were coded and transferred to IBM cards for statistical analysis. As for the Genetic Program data, the statistical analysis has drawn heavily on approaches which make allowance for certain uncontrollable sources of bias. The first results consist primarily of comparisons of the different groups of children with respect to a wide variety of characteristics with particular reference to the degree of inbreeding. Beyond this, however, it is anticipated that the material will permit tentative conclusions concerning the number of "lethal-equivalents" carried by the average Japanese, utilizing a

Part A (continued)

mathematical approach similar to that suggested by Morton, Crow, and Muller (1956).

A major portion of the statistical analysis is now completed. This has, for the most part, been accomplished with the use of high speed digital computers (IBM 704, 709, 7090). Aspects of the data for which analysis is essentially complete include: comparability of the inbred classes (socio-economic variable, parental age, birth rank, migration, seasonal differences in time of examination, etc.), medical history, mortality, physical examination, dental examination, anthropometry, and laboratory findings. Data from the psychometric examination and that relating to school performance have not been completely analyzed at this time.

Analysis of the continuous variables has relied heavily on multivariate regression techniques. In the case of the categorical variables, both an exponit and a logistic function have been used to describe the relationship between inbreeding and the attribute in question, and the fit to both models has been explored for each attribute as well as tests for heterogeneity between inbred classes, sexes, and cities.

Major Findings:

Any attempt to even summarize the overall findings to date would, of necessity, be quite extensive. Since only the oral aspects of the study were the immediate concern of this investigator, this report will be limited to that area. For a summary of the final sample as well as a brief description of the findings in regard to morbidity and mortality, see Neel and Schull, Proc. Nat. Acad. Sci., 48:573-582, 1962.

Inbreeding Effects on Dental Traits:

The oral aspects may be divided into four main categories: gross pathology, including periodontal disease (primarily gingivitis); malocclusion, including dental growth and maturation; dental caries rates; and anomalies of tooth development, including structural defects of dental tissues.

1. a. Gross Pathology (hare lip, cleft palate, tumors, severe infection, etc.): In a population sample of this size (6000-7000) the number of major defects and diseases involving the oral cavity would be expected to be relatively small. Less than 30 affected children were found; obviously a study of consanguinity effects in such a small group is unwarranted. Accordingly, this group was included with

Part A (continued)

those having other major defects for analysis of inbreeding effects (Neel and Schull, 1962).

b. Gingivitis: Significant sex and city heterogeneity exists. Although there appears to be an indication of increased periodontal difficulties with increased inbreeding, the results are inconclusive.

2. a. Malocclusion: A consanguinity effect appears to exist, with inbred children having more malocclusion than outbred.

b. No consanguinity effect was noted for dental eruption status.

3. Dental Caries: No inbreeding effect was noted.

4. Tooth Anomalies: No relation between inbreeding and congenitally missing or supernumerary teeth was found. The findings are inconsistent for enamel defects.

Other Dental Findings:

1. Abnormalities of tooth number, both supernumerary and congenitally missing were more prevalent in Japanese than previously reported for other populations.

2. Notable sex differences exist in both anomalies.

3. No clear-cut, simple mode of inheritance can be established for supernumerary teeth.

4. Positive associations were found to exist between various tooth anomalies, e.g., between congenitally missing teeth and hypoplastic teeth; also between fused deciduous teeth and missing permanent teeth.

5. There is evidence that an association exists between size of the dentition and predisposition to "missing" or "extra" teeth.

6. Contrary to some reports in the dental literature, no association could be found in these data between febrile disease in early childhood and enamel defects of the teeth. The same applies to allergic manifestations and enamel defects.

See previous reports for additional findings.

Significance to Dental Research:

1. Properly executed studies on consanguinity effects will yield

Part A (continued)

information on the genetic structure of human populations which at present can be obtained in no other way. Such information is vital to a realistic, quantitative appraisal of such problems as are posed by the increasing exposure of the human species to ionizing radiation.

2. The Japanese Study will offer an opportunity to study the genetic effects of a specific type of inbreeding, complementing our Brandywine Study. Whereas the latter program is assessing the effects of continuous inbreeding, the Japanese Study has the unique advantage of affording an evaluation of one type of mating pattern.

3. It would be expected that certain recessive characteristics determining normal growth and development, as well as pathological traits, would show up in the homozygous state with increased frequency. Many of these conditions are probably unknown, and the Japanese Study consequently offers an opportunity to define both normal factors and pathological traits. In addition, many of these traits are undoubtedly polygenic and must be studied statistically. These data offer an excellent opportunity for such studies.

4. The Brandywine population group is descended from relatively few ancestors. Consequently, unless an ancestor carried a hidden trait, it could not appear in the descendants, that is, barring mutation. Therefore, the Brandywine group would present only those inherited conditions carried by predecessors. On the other hand, the first cousins in Japan are drawn from a large unrelated population, and therefore, many characteristics and pathological traits should appear in their offspring.

5. In addition to the study of inbreeding effects, the normative aspects of the data offer numerous opportunities for the investigation of the role of racial and environmental factors in many oral conditions.

Proposed Course of Project:

The proposed date for completion of the consanguinity study (i.e., final manuscript of monograph) is December, 1963. This monograph will include one chapter on the dental findings. In addition, a number of other studies utilizing these data are planned. We are presently undertaking a study of the association between the head, maxillo-facial complex, and the rest of the body with respect to growth processes.

Part B included Yes

Serial No. NIDR-71 (C)
1. Clinical Investigations
2. Human Genetics
3. Bethesda, Maryland

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Part B Honors, Awards, and Publications

Publications Other than Abstracts from this Project:

Niswander, J. D., and Sujaku, C.: Dental Eruption, Stature, and Weight of Hiroshima Children. J. Dent. Res. 39:959-963, 1960.

Niswander, J. D., and Sujaku, C.: The Relationship of Enamel Defects of Permanent Teeth to Retention of Deciduous Tooth Fragments. J. Dent. Res. 41:808-814, 1962.

Niswander, J. D.: Effects of Heredity and Environment on the Development of the Dentition. Publications of the American Association for the Advancement of Science, 1961 (in press).

Niswander, J. D., and Sujaku, C.: Congenital Anomalies of Teeth in Japanese Children. Submitted to the American Journal of Physical Anthropology.

Schull, W. J., and Neel, J. V.: The Child Health Survey: A Genetic Study in Japan. In: UN/WHO Seminar on the Use of Vital and Health Statistics for Genetic and Radiation Studies, Sept. 5-9, 1960, Geneva, New York, 1962. pp. 171-193.

Schull, W. J., and Neel, J. V.: The Effect of Inbreeding on Mortality in Japan. Second International Conference on Human Genetics, Rome, 1961 (in press).

Neel, J. V., and Schull, W. J.: The Effect of Inbreeding on Mortality and Morbidity in Two Japanese Cities. Proc. Nat. Acad. Sci. 48: 573-582, 1962.

Honors and Awards Relating to this Project: None.

Serial No. NIDR-72 (C)
1. Clinical Investigations
2. Human Genetics
3. Bethesda, Maryland

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Part A

Project Title: Tri-racial Isolates in Eastern United States

Principal Investigator: Dr. C. J. Witkop, Jr.

Other Investigators: None

Cooperating Units: National Institute of Arthritis and Metabolic Diseases

Man Years (calendar year 1962):

Total:	1/4
Professional:	1/4
Other:	None

Project Description:

Objectives:

The purpose of this investigation is to find, enumerate and describe all tri-racial isolates residing in Eastern United States; to investigate the degree of consanguinity in these groups; to look for unusual types of matings, rare in human material, that are of special value in determining modes of inheritance, gene interaction, and biochemical pathways; to investigate the hereditary diseases present in these groups; and to utilize the patient material found for further clinical and biochemical genetic investigations. It is assumed that in isolate populations consanguinity will be prevalent; and it is further assumed that an increased number of individuals in such a population will show homozygous recessive illness. It is also assumed that all cases of a specific hereditary illness from any one group will nearly always be due to the same gene defect, thus providing an epidemiological method for selecting a large sample of individuals with the same gene defect for biochemical investigations in other NIDR projects or other Institutes.

Methods Employed:

This investigation requires three approaches:

Part A (continued)

1. Analysis of census and other records:

Because of the U. S. Census Bureau's wish to further classify people whose race entries were somewhat nebulous, the 1950 Enumerators Reference Manual directed, "Report persons of mixed white, Negro, and Indian ancestry living in certain communities in Eastern United States in terms of the name by which they are locally known." Such persons were then to be classified for publication purposes among "other nonwhite races;" that is, other than Negro, American Indian, Chinese, Japanese, or Filipino. Starting with these data, and tracing the surnames so recorded through previous U. S. and British Colonial Census records, it was determined that such surnames frequently residing in the same household or neighborhood showed various race entries from decade to decade, even for the same individual (i.e., white, Negro, Indian or other), and for the most part had large families. The existence of suspected tri-racial isolates from these data were then verified from local civil, historical, and social records, such as marriage records, historical accounts, and newspaper articles. A search of the anthropological and social literature frequently revealed studies on such groups. Visits to these communities, to verify the existence and surname data, were made by collaborators and this investigator.

Of the 116 counties checked, the population of tri-racial character was estimated at 77,000 persons, of which 33,000 were listed as Indian, 29,000 as white, 14,000 as Negro and 1,000 under colloquial race names. Forty percent of the total reside in North Carolina.

Local and census records were used to estimate consanguinity and fertility. For example, census data from one North Carolina group showed the highest fertility ratio for any known racial or ethnic group in the United States. The ratio of children under 5 years old per 1,000 women 15 to 49 years old was 825, as compared to 417, the U. S. average. Marriage records of one group showed that 177 required ecclesiastical dispensation for relationship within the first three degrees (i.e., 2nd cousins or closer). Investigation of the marriage records in Barbour and Taylor Counties, West Virginia, showed that 102 of the 112 marriages from 1856 to 1931 were to other tri-racial group members. School records of the same group in 1958 showed an unusual proportion of students from the group in the higher age levels for the grade in which they were placed.

2. Survey of groups by letters to physicians and public health officials:

Part A (continued)

After determining the number and surnames of persons comprising these isolate populations, letters were sent to all county health officials in the 116 counties where these people reside requesting information on specific listed hereditary illnesses among these groups and any illness that appeared unusually frequent in such populations. Other local physicians and dentists were also contacted. These returns were tabulated. Literature by all writers was scanned for mentioned hereditary illness in these groups, (e.g., microphthalmia in the Moors of Delaware).

3. Field work by NIDR team:

Biological specimens are collected in the field for analysis at the National Institutes of Health. These include bloods, urines, exfoliative cytology smears and biopsy material. In general, this work has been directed along two lines: (1) determining blood group frequencies, linkages and other biochemical or cellular abnormalities of blood fluid and urine; (2) determining the genetic patterns of inheritance of two particular diseases - hereditary benign intraepithelial dyskeratosis and oligophrenia, spasticity and ichthyosis. This latter condition is inherited as an autosomal recessive trait. The clinical features of this latter condition have been recorded in a movie film.

Patient Material:

Patients found in this study of isolates have been admitted from time to time for the study of particular diseases. In addition to the diseases appearing in isolates, it was found necessary to admit certain families from the general population suffering from similar diseases in order to make cytological and cytochemical comparisons of these hereditary defects.

Major Findings:

1. Hereditary benign intraepithelial dyskeratosis was shown to be inherited as an autosomal dominant trait and affects oral mucosa and bulbar conjunctiva, leading to blindness. This new hereditary disease has been described, and publication was made late in 1960.

2. Comparison between the cellular, the cytochemical and the genetic findings in hereditary benign intraepithelial dyskeratosis was made with pachyonychia congenita of Jadassohn and Lewandowsky, white sponge nevus of Cannon, and Darier's disease. It has been determined that Darier's disease, hereditary benign intra-epithelial dyskeratosis and white sponge nevus of Cannon are

Part A (continued)

characterized by diagnostic discrete individual cell lesions. A new method of diagnosis of these conditions has been worked out by the exfoliative cytology technique. These methods have been described in the A.M.A. Archives of Dermatology and in a scientific exhibit presented at the annual session of the American Academy of Dermatology and Syphilology, Chicago, December 1-7, 1961.

3. The study of the dyskeratosis led us to theorize that the bases for these cellular abnormalities were genetic defects in the synthesis of nucleic acid. It has been found that patients receiving Methotrexate, 5-Fluorouracil, 5-Fluorodeoxyuridine, have similar cell lesions induced by these drugs in normal buccal mucosal cells. This reaction takes place within three days after administration of the drugs. It is postulated that either genetic or environmental factors which interfere with nucleic acid synthesis will induce this cellular change. This cellular change is manifested by a defect in cytokinesis and abnormal division of the chromosome and the formation of peculiar dyskeratotic cell-within-cell bodies. These findings were presented at the First International Congress of Exfoliative Cytology, Vienna, and are now published in book form by Lippincott Company, Philadelphia.

4. Cytological methods have now been worked out for the rapid smear technique to assist in the diagnosis of a number of non-neoplastic oral lesions including pemphigus, hereditary benign intraepithelial dyskeratosis, white sponge nevus, Darier's disease, herpes, and PPLO infections of the periadenitis mucosa necrotica recurrens type.

5. Investigation of 28 examples of the neurological defect present in the Haliwa Indians was made by admission of patients to the National Institutes of Health and by recording the findings in a motion picture. The clinical features of this disease are oligophrenia, spasticity, ichthyosis of the skin of the trunk, and an abnormal pigmentation of the retina. This condition is inherited as an autosomal recessive trait, and all 28 affected individuals are the offspring of consanguineous matings. In addition to the primary symptoms listed, the affected individuals show: (1) loss of sensation in the anterior oral cavity, (2) loss of tactile sensation in the tongue, (3) loss of normal masticatory function, (4) a speech defect, and (5) aminoaciduria.

   A study of the high histidine excretion of these patients, combined with the finding of a speech defect in 9 out of 10 cases of histidinemia, substantiates these two conditions,

Part A (continued)

Sjogren-Larsson syndrome and histidinemia, as the first defined biochemical defects affecting speech and oral functions.

Significance to Dental Research:

1. Biochemical defects affecting speech and mastication have been defined for the first time.

2. A rapid cytological method to aid in the diagnosis of a number of non-neoplastic oral diseases has been perfected and the characteristic cellular changes described.

3. Clinical application of these findings will result in better diagnosis and treatment of these oral disorders.

Proposed Course of Project:

To continue to accumulate information on the hereditary diseases present in these isolate populations. To create two new projects: (1) Hereditary Biochemical Defects of Speech and Hearing and (2) Exfoliative Cytology of the Oral Mucosa. Screening of these populations for new leads on genetic diseases will be continued. Projects will be developed around those that offer research possibilities.

Part B included Yes

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Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Witkop, C. J., Jr.: Intraepithelial cell bodies associated with hereditary dyskeratoses and cancer therapy. In Proceedings of the First International Congress of Exfoliative Cytology. Lippincott, Philadelphia, 1962.

Honors and Awards Relating to this project: None.

Serial No. NIDR-73 (C)
1. Clinical Investigations
2. Human Genetics
3. Bethesda, Maryland

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Part A

Project Title: Genetic Study in Southern Maryland

Principal Investigator: Dr. C. J. Witkop, Jr.

Other Investigators: Dr. R. O. Wolf, H. R. Dyson, R.N., Dr. C. S. Chung, Dr. N. Brown, Dr. I. Zipkin

Cooperating Units: School of Dentistry, Howard University;
Sociology Department, Catholic University;
Dermatology Branch, N.C.I. and N.I.A.M.D.

Man Years (calendar year 1962):

Total:	3-3/4
Professional:	1-3/4
Other:	2

Project Description:

Objectives:

1. To determine the total amount of hereditary illness present in an isolate population of not over 5,000 related individuals of white, American Indian and Negro ancestry as revealed by dental and physical examinations and selected laboratory procedures.

2. To determine the prevalence, mode of inheritance, effects on viability and longevity, clinical manifestations and variations in expressivity of the hereditary illness found.

3. To investigate the relationships and possible linkages between these illnesses and blood types, secretor factor, P.T.C. taste test and other genetic markers.

4. To investigate genetic factors in illness not usually known to have a hereditary component.

5. To select patient and biological material from this population for clinical and biochemical investigations of specific illnesses and normal factors (these will constitute separate projects).

Part A (continued)

6. To assess the effects of inbreeding as revealed by in-group and out-group marriages and by degree of relationship (i.e., first cousins, first cousins once removed, second cousins, etc.) as shown by perinatal deaths, viability, longevity, and the presence of apparent homozygous recessive illness.

7. To try to trace the source of outflow of pathological genes in this population.

8. To assess the social and genetic factors in mate selection.

9. To look for correlations between genetic illnesses and other normal and abnormal factors that may have etiological or diagnostic relationships.

10. To try to reconstruct, on the basis of gene frequencies, the source of the group's genetic material.

11. To compare the prevalence of illness, gene frequencies, and vital statistics with other isolate populations and the general population.

12. To detect carriers of recessive genes, if possible.

Methods Employed:

This study is divided into three phases:

1. Field study:

The field study consists of history and dental, medical and laboratory examinations designed to detect as much hereditary illness as possible.

a. History, genetic and census data are obtained on a questionnaire covering 158 items which arranges the data by family groups and by sibships. Such items as birth order effects, parental age, consanguinity, age of onset, longevity and perinatal mortality are included. A record of hospitalizations and institutionalizations is obtained in addition to a medical and genetic history. A master kindred chart showing the relationship of each individual is made.

b. A search of existing social and church records for births and marriages is made by Fr. Thomas Harte of Catholic University. Sociological data on development in marriage patterns in this group are also obtained.

Part A (continued)

c. All subjects in this study are given a complete dental and medical examination, designed to detect as much hereditary illness as possible. This includes complete blood typing genetic markers; urinalysis; hematological work-up, especially for abnormal hemoglobins; and special procedures, such as X-rays and ophthalmological examinations, where indicated. These examinations are made during the summer months at mobile units.

d. All data are coded and tabulated for I.B.M. punch cards.

2. Clinical Study:

Selected patients seen in the field study are brought to NIH for detailed clinical studies. These studies are designed to give a description of the hereditary diseases involved; to determine the limits of the signs and symptoms of the illness; and to describe any metabolic abnormalities detectable on routine laboratory examinations, or other associated defects that might be found on X-rays or by special procedures.

3. Biochemical Investigations:

Selected genetic defects are being investigated biochemically to find the chemical error present. Dentinogenesis imperfecta is being investigated histochemically and by chemical analysis for abnormal protein constituents. Histidine metabolism in patients with untreated pernicious anemia and liver disease was investigated to help define this biochemical pathway and the alterations found in these diseases. This study led to the investigation of histidine utilization in several conditions demonstrating speech defects (See Project D4-010).

Patient Material:

Patient material is selected from the families seen in the field studies. Approximately 50 hospital days have been utilized by this Institute.

Major Findings:

1. A biochemical investigation of albinism was conducted. This study utilized the albinos found in the isolate, as well as albinos collected from the general population, in review of the following:

Part A (continued)

a. That most albinos do have tyrosinase activity in the melanocytes of the skin and hair bulbs.

b. These albinos have normal sera levels of tyrosine.

c. By stripping off the keratin layers of the skin with repeated Scotch tape strippings and by packing the skin with wet packs of tyrosine, pigmentation can be induced in albinos.

d. Loading experiments with hair-test positive (tyrosinase positive) albinos did not induce pigmentation where sera levels of 20 mg.% were maintained for 24 to 48 hours.

e. The hair-test-positive albinos have the inability to secrete normal levels of tyrosine in the saliva. This work was done in cooperation with Dr. Zipkin.

f. Pigmentation cannot be induced by incubating skin and hair in certain types of albinos.

g. The net results of these studies indicate that there are two types of recessive albinism in man, and that the biochemical defects in each are different. The two types of albinos (i.e., the hair-test positive albinos and the hair-test negative albinos) have not occurred in the same sibship, to date. From this we postulate that one type of albinism is due to a defect or an absence in the enzyme tyrosinase. This is by far the rarer type of albino. The other type of albinism does possess active functional tyrosinase. The defect in this type appears to be in the transport mechanism between the normal sera levels and the tyrosinase-intact melanocyte. It is postulated that this is a defect in a permease or a transferase.

2. Urocanic acid has been definitely identified as a urinary metabolite in man. Methods for the quantification of urocanic acid and α-formiminoglutamic acid in human urine were described. Both urocanic and α-formiminoglutamic acid excretion are increased after oral histidine loading in normal control subjects and in patients with hepatocellular disease. The response to loading in patients with hepatocellular disease is exaggerated.

Significance to Dental Research:

An isolate population has been found containing a vast number of

Part A (continued)

hereditary diseases which may shed light on genetics and biochemical pathways of various hereditary defects. Indications have been obtained that some speech defects may be the result of genetically determined biochemical blocks.

Proposed Course of Project:

1. Blood group data and dental data on 3,506 persons have been programmed and initially processed.

2. Papers on the findings in this study are in preparation.

Part B included Yes

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Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Witkop, C. J., Jr.: Dental problems of a heritable nature, p. 42-56. (In Witkop, C. J., ed. Genetics and Dental Health, N. Y., McGraw-Hill, 1962).

2. Witkop, C. J., Jr. (ed.): Genetics and Dental Health. N. Y., McGraw-Hill Book Co., Inc., 1962. 300 p.

3. Witkop, C. J., Jr., Van Scott, E., and Jacoby, G. A.: Evidence for two forms of recessive albinism in man. Proc. First Internat. Cong. Human Genetics, Rome, 1962.

4. Merritt, A. D., Rucknagel, D. L., Silverman, M., and Gardiner, R. C.: Urinary urocanic acid in man: The identification of urocanic acid and the comparative excretions of urocanic acid and N-formiminoglutamic acid after oral histidine in patients with liver disease. J. Clin. Invest. 41:1472-1483, 1962.

5. Chung, C. S., and Witkop, C. J., Jr.: Genetics in Dentistry. In Provenza, V. (ed.), Oral Histology and Embryology. Philadelphia, Lippincott, 1962 (in press).

6. Hanna, B. L., Sawin, P. H., Sheppard, L. B.: Recessive buphthalmos in the rabbit. Genetics 47:519-529, May, 1962.

7. Hanna, B. L.: Family studies of the facial complex. Proc. Symp. on Dental Genetics. AAAS, Denver, 1961. J. Dent. Res. (in press).

8. Witkop, C. J., Jr.: Recent advances in dental genetics. Proc. Symp. on Dental Genetics. AAAS, Denver, 1961. J. Dent. Res. (in press).

Honors and awards relating to this project: None.

Serial No. NIDR-74 (C)
1. Clinical Investigations
2. Human Genetics
3. Bethesda, Maryland

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Part A

Project Title: Genetic Studies in a Population Isolate (Brandywine) with Particular Reference to Hemoglobin and Haptoglobin Patterns

Principal Investigators: Dr. C. J. Witkop, Jr.

Other Investigators: Dr. A. D. Merritt, Mr. R. Robinette, Dr. L. Schneiderman

Cooperating Units: Blood Bank, Clinical Center, NIH

Man Years (calendar year 1962):

Total:	1/4
Professional:	1/4
Other:	None

Project Description:

Objectives:

1. To utilize the haptoglobin and hemoglobin patterns within the population isolate as markers for the study of disease processes and other biologic data available from these patients.

2. To identify unusual hemoglobin and haptoglobin patterns and to define the types of haptoglobins produced when analyzing sibships in this population.

Methods Employed:

Sera and hemoglobin were collected from the Brandywine population isolate and studied by means of starch gel and paper electrophoresis respectively. The genetic types of haptoglobin and hemoglobin are being defined and will be analyzed by standard statistical methods. Differences in the peptides of fetal and adult myoglobin were sought by the peptide method ("fingerprinting").

Part A (continued)

Patient Material:

The patient source material for this study is derived from a major triracial isolate situated in the Brandywine, Maryland, area. Most of the samples have been collected in field studies.

Major Findings:

Sickle cell hemoglobin is found in approximately 20% of the population (national average approximately 10% in a Negro population) and 2-1 "modified" haptoglobin patterns have been found in a correspondingly increased percentage. The progeny from 2-1 "modified haptoglobin parents are being defined.

Fetal myoglobin is indistinguishable from adult myoglobin by the two-dimensional peptide "fingerprint" technique.

Significance to Dental Research:

These genetic markers will serve for a correlation point for other disease states, and abnormal and normal findings within this triracial isolate. The general significance lies in the further definition of the pattern by which man inherits his normal and abnormal characteristics and the possible influence of genetically determined traits, one upon the other.

Proposed Course of Study:

This project is discontinued.

Part B included Yes

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Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Merritt, A. D., Witkop, C. J., Jr., Robinette, R., and Schmidt, P. J.: The serum haptoglobins (Hp) in a tri-racial isolate. Proc. 2nd. Internat. Cong. Human Genetics, Rome, Sept. 1961. Excerpta Med., London, 1962, pp. 153.

Schneiderman, L. J.: Myoglobin in the human foetus. Nature 194:191-192, April 14, 1962.

Honors and Awards Relating to this Project: None.

Serial No. NIDR-75 (C)
1. Clinical Investigations
2. Human Genetics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
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Part A

Project Title: Sickle-cell Anemia, Other Inherited Hematological Factors and Blood Groups Related to Oral Disease.

Principal Investigators: Dr. C. S. Chung

Other Investigators: Dr. C. J. Witkop, Jr., Dr. J. Moor-Jankowski, Mr. W. C. Leyshon, Mr. R. Robinette

Cooperating Units: Department of Human Genetics, University of Michigan

Man Years (calendar year 1962):

Total:	3/4
Professional:	1/2
Other:	1/4

Project Description:

Objectives:

(1) To study the relationships between certain blood cellular and serological characteristics and oral disease, especially as it relates to linkage or other relationships.

(2) To study population dynamics of blood groups, hematological disorders, and other genetic factors in human populations and compare these discretely inherited traits with less well-defined (polygenic) oral and medical traits as to effects of inbreeding or outcrossing.

(3) To investigate the oral consequence of various serological incompatibilities and to use known tooth defects to date intra-uterine damage.

(4) To develop the methodology of analysis of population data best adapted to the modern computer (Honeywell 800).

(5) To seek new genetically determined serological systems (isoantigens) by studies of multitransfused human material and immunized primates.

Part A (continued)

Methods Employed:

(1) Data collected on 3,506 individuals from the Brandywine study, including over 300 complete families, have been processed, coded and punched, and the format for analysis on the Honeywell 800 has been established.

(2) These data will be subjected to gene frequency, segregation (SEGRAN), and multivariate analysis to study the population dynamics and interactions involved.

(3) Inbreeding coefficient to be calculated will be correlated with various abnormal and normative dental and medical data to test for genetic variability.

(4) Chilean and other population survey data will be analyzed in a similar manner where applicable.

(5) Laboratory data from serological testing, electrophoresis and isoantigenic studies from man and primates will be analyzed in a similar manner where applicable.

(6) These data from all sources will test:
- (a) Relation of blood factors and presence of oral disease.
- (b) The relative magnitude of change of dental traits with in and out breeding compared to discrete single gene factors.
- (c) A reciprocal sample will be drawn from the dental defects to be investigated for potential serological incompatibilities or cell abnormalities.

Patient Material:

Patient material is derived from other field projects of the Section and include 3,506 biological samples from the Brandywine study, 673 samples from Chileans, 140 samples from Araucanian Indians and 500 samples from miscellaneous populations. Sera for isoantigen studies are obtained from various laboratories at NIH and universities.

Major Findings:

1. The computer program on the Honeywell 800 for SEGRAN has been completed and tested. Statistically this is found to be a most efficient method for this type of analysis. This method is extremely sensitive to segregation distortion which may be due to genetic selective forces.

Part A (continued)

2. The formulation for gene frequency analysis by the efficient method of maximum likelihood has been completed and part of the computer program has been checked out.

3. A statistically significant excess of people with scrotal tongue are blood group O.

4. Chileans with scrotal tongue have a statistically significant mean serum vitamin A level. This relationship does not obtain for fissured tongue.

5. The gene frequencies for blood groups of Araucanian Indians and Chileans have been calculated and reported at the International Hematological Congress in Mexico City. They are being prepared for publication.

6. There was no correlation between periodontal index scores or DMF rates and blood groups ABO, MN and Rh in the Chilean population.

7. There was no correlation between blood groups ABO, MN and Rh and vitamin deficiencies in the Chilean population.

8. A significant excess of persons with enamel hypoplasia has been found in sibships from ABO and Rh incompatible matings.

9. A baboon isoantigenic system that cross reacts with some but not all baboon sera and with some but not all human sera has been found.

10. The claim for specificity of the Rh hump on deciduous incisors to those with kernicterus has been disproved and a series of five families have been investigated wherein this defect is associated with A-O incompatibility without serious neurological damage.

Significance to Dental Research:

A number of parental serological incompatibilities are known to lead to damaged enamel. In one of our studies, the hypothesis appears to hold that given enamel defects it will be found that an excess of these children will be from A-O, Rh incompatible matings. The role of these relationships is being investigated further and possible minor neurological damage, i.e. minor speech and reading defects, is being investigated.

Part A (continued)

Proposed Course of Projects:

This project will be split into two future projects: 1. The Montgomery County Study of Blood Group Incompatibilities and 2. the present study.

Part B included Yes

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Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Chung, C. S.: Genetic analysis of human family and population data with use of digital computers. Proc. 3rd IBM Med. Symp. 51-78, 1962.

Moor-Jankowski, J.: Immune hetero- and iso-antigenic systems in sera of primates. Proc. of Symp. on Primates. (in press).

Honors and awards relating to this project: None

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Serial No. NIDR-76 (C)
1. Clinical Investigations
2. Human Genetics
3. Bethesda, Maryland

PHS-NIH
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Part A

Project Title: Hereditary Defects of Enamel and Dentin

Principal Investigator: Dr. C. J. Witkop, Jr., Dr. L. Barros

Other Investigators: None

Cooperating Units: ICNND

Man Years (calendar year 1962):

Total:	2-1/4
Professional:	1-3/4
Other:	1/2

Project Description:

Objectives:

1. To define the various hereditary defects in enamel and dentin.
2. To determine the prevalence and mutation rates in the general population.
3. To determine the mode of inheritance.
4. To investigate the histological processes involved in the defects.
5. To describe any associated physical or chemical defect.
6. To investigate possible linkage associations.
7. To determine the various methods of restoration, prevention or treatment, where possible.

Methods Employed:

1. Field Study: A survey of 96,761 school children between the ages of 4 and 12 was conducted in 42 counties in the state of Michigan for hereditary defects and other formations. Subsequent

Part A (continued)

pedigree studies on these individuals were conducted to determine the mode of inheritance and possible linkage with known genetic markers such as blood type, secretor factor and P.T.C. taste test. These defects were classified by clinical descriptions, histological findings and inheritance patterns. Patients with representative difficulties were brought to NIH for clinical evaluation.

2. Clinical Study: Complete physical and routine laboratory examinations were given to determine if any physical or metabolic defect was also associated with the tooth defect. Except in one particular defect, osteogenesis imperfecta, no genetic defect was found associated with the tooth condition.

3. Laboratory Study: Histochemical studies conducted on fresh ground and decalcified specimens, using normal controls, are utilized to determine the possible biochemical defect in opalescent dentin. Linkage studies were analyzed.

4. In 1960 the ICNND Nutritional Survey of Chile included these investigators on its team. Results of the dental investigations on 1,906 Chileans will be reported under this project.

Patient Material:

Patient material was obtained from: (1) individuals contacted during our field study in Michigan; (2) individuals in our Genetic Study in Southern Maryland, Project No. D-4-002; and (3) in a few instances, from individuals referred by local practitioners.

Major Findings:

1. The tooth defect in a rare form of vitamin D-resistant rickets was found to consist of marked interglobular dentin formation and microfissures from pulp to enamel. This results in bacterial invasion and abscess formation in clinically normal-appearing teeth. The presence of multiple abscesses on clinically normal-appearing teeth may be one of the first signs of this disease.

2. Based on the findings in northern Chile, a revised formula for calculating optimal fluoride concentrations allowing for variations in mean maximum temperatures and milk consumption has been proposed:

$$\text{Optimum fluoride in ppm} = \frac{.34}{E}$$

Part A (continued)

$$E = \frac{(-0.038 + 0.0062T)\ W}{.56}$$

where E is the estimated water consumption in ounces per pound of body weight and W is the average proportion of domestic water in the total daily fluid intake of children 0 to 10 years of age, and T is the mean maximum temperature in degrees Fahrenheit averaged over at least five years.

3. The dental treatment requirements for a representative sample of Chilean military Army, Air Force and Navy personnel have been determined, giving these services their first epidemiologically based estimates of these needs. A representative 1,000 man unit was found to require 2,096 fillings, 1,047 teeth needing extraction, 942 men needing prostheses, 499 men needing routine periodontal care and 159 men needing advanced periodontal care. Proposals concerning training of dental assistants and hygienists to assist in meeting these needs were made.

4. Periodontal disease in Chilean civilians and military in relation to nutritional status was analyzed by direct comparison and multivariate techniques. Vitamin deficiency or low vitamin levels contribute negligible variance in mean PI scores. Local factors such as the presence of oral debris and calculus contributed over 70% of the variance in mean PI scores. Age, gingival recession, and attrition contributed a minor but significant variance in periodontal index scores. These data do not substantiate the idea that nutritional status contributes significantly to the common forms of periodontal disease.

5. Mutation rates have been estimated for the average gonadal dose from a full-mouth series of X-rays.

Significance to Dental Research:

1. A formula for estimating optimum fluoride levels in domestic waters if substantiated by future studies would indicate that many communities living under high mean maximum temperatures and consuming little fresh milk would need much less (.4 to .6) ppm of fluoride to achieve the same biological effects as U. S. cities at 1.0 to 1.2 ppm of fluoride.

2. Basic data for planning dental treatment programs in the Chilean military have been presented.

3. Research on periodontal disease should be directed toward factors in oral debris and calculus, as the most immediate practical control procedures seem to evolve around elimination of these factors.

Part A (continued)

4. The dental aspects of one form of hereditary vitamin D-resistant rickets have been described.

5. A popular monograph on Genetics has been prepared for high school students.

6. Assessment of the genetic risks for dental X-rays has been made.

Proposed Course of Project:

Completion of analysis of data and preparation of manuscripts for publication. Biochemical defects in the hereditary tooth defects will continue to occupy a portion of our research effort. Four manuscripts have been accepted for publication and are now in press.

Part B included Yes

Serial No. NIDR-76 (C)
1. Clinical Investigations
2. Human Genetics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
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Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Witkop, C. J., Jr.: Dental Genetics. In Paffenberger, G. C., Pearlman, S., and Brown, W. R. (eds.): Frontiers of Dental Science. N. Y., Scholastic Book Services, Dec., 1962, pp. 48-64.

2. Witkop, C. J., Jr., Barros, L., and Hamilton, P. A.: Oral and genetic study of Chileans, 1960. Geographic and nutritional factors in dental caries. Public Health Reports 77(11):928-940, Nov., 1962.

3. Witkop, C. J., Jr.: Enamel defects. J.A.M.A. 182:223, Oct. 13, 1962.

4. Witkop, C. J., Jr., and Barros, L.: Oral and genetic study of Chileans, 1960. II. Oral anomalies. Am. J. Phys. Anthrop. (in press).

5. Barros, L., and Witkop, C. J., Jr.: Oral and genetic study of Chileans, 1960. III. Periodontal disease and nutritional factors. Arch. Oral Biol. (in press).

6. Barros, L., y Witkop, C. J., Jr.: Estudio oral y genetico de la población chilena, 1960. IV. Necesidades de tratamiento dental en las fuerzas armadas. Revista dental de Chile (in press).

Honors and awards relating to this project: None

Serial No. NIDR-77 (C)
1. Clinical Investigations
2. Human Genetics
3. Bethesda, Maryland

PHS-NIH
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Part A

Project Title: Discrimination and Segregation Analysis of Hereditary Deafness in the Students of the Clarke School for the Deaf

Principal Investigators: Dr. K. S. Brown, Dr. C. S. Chung

Other Investigators: Dr. C. J. Witkop, Jr., Dr. E. Graykowski, Mr. R. W. Robinette, Mr. W. C. Leyshon

Cooperating Units: The Clarke School for the Deaf, Northampton, Mass.; National Institute of Neurological Diseases & Blindness; Bionetics Research Laboratories, Falls Church, Virginia (under contract).

Man Years (calendar year 1962)

Total:	2-1/4
Professional:	1-1/2
Other:	3/4

Project Description:

Objectives:

To discriminate the various types of deaf people into groups based on the characteristics of their conditions, including patterns of audiogram, physical findings and laboratory values. To analyze the patterns of genetic segregation among the various types of hereditary deafness in order to estimate the number and kind of genetic factors causing deafness.

Methods Employed:

Personal examination of about two hundred fifty deaf people and two hundred eighty of their hearing relatives including parents, grandparents and siblings has been completed. The examination has been limited to the head and neck, except for blood pressure and cardiac auscultation. It has included caloric vestibular examination, oral examination with DMF, otoscopic and ophthalmoscopic examinations of 170 deaf children who are currently attending the Clarke School and a similar examination lacking the caloric and dental examinations, but including a pure tone audiogram for the hearing relatives. All

Part A (continued)

but the youngest children were given a PTC taste test, a routine microscopic urinalysis, and urine analysis for glucose, protein, and phenylketone-like substances.

Blood samples were drawn for genotyping and laboratory studies of the serum. These will include protein and lipoprotein electrophoresis, total protein, total lipid, and cholesterol determinations. All individuals showing clinical goiter will be examined for serum butanol extractable iodine. A contract has been negotiated with Bionetics Research Laboratories, Inc., by NINDB to carry out the serum studies exclusive of the electrophoreses.

The pedigree and history material collected by the Clarke School has been examined, and a questionnaire form to supplement and update it has been prepared (Budget Bureau No. 68-6229), and over 2,000 copies have been distributed. The total genetic information resulting is being coded by employees of the Clarke School under our direction on two computer coding sheets prepared for this purpose (PHS-T-96, PHS-T-97). This work is being done under a contract between the Clarke School and NINDB. The resulting coded data will then be processed at the NIH computer center, utilizing the SEGRAN program, developed by Dr. Chung.

Patient Material:

Patient material consists of the students at the Clarke School for the Deaf and their siblings and parents or other relatives, if indicated. The alumni of the Clarke School and their families, where possible, will be examined, including as many of the graduates since 1930 as are still available. An estimated 1,500 people are available, of whom about 700 are deaf.

Major Findings:

Clinical thyroid abnormality occurs in about 10% of the adult alumni. This includes history of thyroid surgery, use of exogenous thyroid medication, and presently palpable goiter. Just over 6% of the current Clarke School pupils, ages 5 to 17 years, had palpable goiter. There is a significant increase in frequency of non-tasters for PTC among the hereditary deaf adults but not among the sporadic deaf. Lack of response to cold caloric vestibular function test is most frequently associated with a history of meningitis.

Significance to Dental Research:

Development of diagnostic criteria which allow the separation of various groups of congenital and non-congenital deafness is basic to the understanding of possible etiologic factors involved. These same

Part A (continued)

factors may be linked to dental and oral development--for example, the finding on taste deficiency.

Proposed Course of Project:

Further examinations of the deaf alumni and their families is planned. The coding of the genetic information is continuing. Laboratory studies of body fluids are to be undertaken. Studies of the thyroid function in goiterous deaf children and adults and their normal relatives is also planned. When the complete data is collected, it will be subjected to statistical analysis.

Part B included No

Serial No. NIDR-78 (C)
1. Clinical Investigations
2. Human Genetics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1962

Part A

Project Title: Saliva Study

Principal Investigator: Dr. R. O. Wolf

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1962):

Total:	2
Professional:	1
Other:	1

Project Description:

Objectives:

1. To use various techniques which have inherent varying resolving powers to determine and define salivary components.

2. To determine the normal variation of the salivary components between normal individuals as related to environmental conditions at the time of collection.

3. To relate components to genetic control. This includes genetic systems such as secretor factor and unknown but suspected control of salivary proteins.

4. After methods are perfected and normal bases are established, the salivary components of selected disease entities will be studied.

Methods Employed:

1. The following methods, some with adaptations, are being or have been investigated to determine the total protein concentration in a given parotid sample: Micro-Kjeldahl, Biuret, Greenburg, Loury, Warburg and Christian, Waddell and a gravimetric method.

2. The following media are being used for the electrophoresis of saliva: paper, cellulose, acetate and acrylamide gel.

Part A (continued)

3. An agglutination inhibition test is used for determining the secretor factor condition of a given saliva sample.

4. Schneyer segregators are individually made for individuals when submaxillary and/or sublingual saliva is desired.

Patient Material:

1. Normal control patients of the Clinical Center (NIH permission for the study of Normal Control Patients has been obtained).

2. Selected Clinical Center patients.

3. Individuals from genetically defined populations.

Major Findings:

1. A method for preventing the loss of the saliva concentrate during the lyophilization procedure.

2. Discrepancies in the amount of protein in a given sample of parotid saliva have been found when two or more different methods of determination are used.

3. Significant differences in the secretor factor concentration between glands have been found.

4. The use of cellulose acetate for the electrophoresis of saliva has not been as satisfactory as has paper.

5. There is considerable variation between individuals as to the electrophoresable components and their concentrations when separated by paper electrophoresis. Experiments are in progress attempting to determine which are environmental and which are genetically controlled.

6. Improvements have been made in the paper electrophoretic techniques for the study of saliva.

Significance to Dental Research:

This study has further elucidated the components, biological control and the significance of this lesser-studied body secretion.

Proposed Course of Project:

We intend to detect and define possible gene-controlled salivary

Part A (continued)

components which, in turn, may lead to the definition of new metabolic pathways or corroborate known ones.

Part B included No

Serial No. NIDR-79 (C)
1. Clinical Investigations
2. Human Genetics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1962

Part A

Project Title: Chromosome and Cell Growth Studies in Normal and Abnormal Subjects

Principal Investigator: Dr. Herbert L. Cooper

Other Investigators: None

Cooperating Units: National Institute of Allergy and Infectious Diseases

Man Years (calendar year 1962):

Total:	1-1/2
Professional:	1
Other:	1/2

Project Description:

Objectives:

1. To study the chromosomes of a series of normal subjects to investigate the frequency of minor chromosomal variants among the normal population.

2. To study the chromosomes of members of families where one or more member has some dental or medical abnormality in an effort to determine the correlation, if any, of the abnormality with chromosomal aberration and to follow the transmission of abnormal chromosomes from one generation to the next.

3. To study the mechanisms of mitotic stimulation in normal cells.

4. To study the chromosomal changes in cells that have undergone malignant degeneration *in vitro* and *in vivo*.

Methods Employed:

Chromosome studies were performed on cultures of lymphocytes obtained from peripheral blood, and on fibroblasts obtained from biopsy material. Lymphocyte cultures were grown by the phytohemagglutinin technique, in which mitotic activity of lymphocytes *in vitro* is stimulated by the addition of kidney bean extract (phytohemagglutinin).

Part A (continued)

Cell growth studies were carried out on lymphocyte cultures, and growth conditions were varied.

For studies of malignant cells, long-term cell cultures of golden hamster cells were used. Some of these have undergone malignant transformation due to infection in vitro by the Simian vacuolating virus.

Patient Material:

Patient material for this study was obtained from the wards of the Clinical Center, from outside referrals, and from the Clinical Center blood bank (normal subjects).

Major Findings:

1. To date, 40 subjects have been studied with particular attention to the morphology of the chromosomal satellite bodies. Approximately one third of these have shown detectable asymmetry of satellite bodies. No other consistent variants have been identified.

2. A family study is in progress in a kindred where several normal and one abnormal member have been found to be carrying an abnormal chromosome number one. This abnormal chromosome can be followed from one generation to the next because of its unique morphology. The presence of this chromosome is being correlated with various blood groups and serum factors in an effort to detect possible linkage to the number one chromosome.

3. Studies are being carried out to determine the nature of the mitotic stimulatory effect known to occur when extracts of the kidney bean are added to human lymphocyte suspensions in cell culture. To this end, a new technique has been devised to obtain relatively pure suspensions of lymphocytes for quantitative studies.

   A method has been devised for doing a reproducible bioassay of the mitotic stimulating properties of phytohemagglutinin (kidney bean extract) so that the threshold dose for any sample of extract can be determined and used for further experimentation.

   Using the minimal dose established by the above-mentioned technique, experiments have been done which demonstrate the existence in normal serum of at least two factors involved in this growth system. One is apparently essential for lymphocyte growth. The other either antagonizes lymphocyte growth directly, or neutralizes the stimulatory effect of the phytohemagglutinin. Further studies are under way in this area.

Part A (continued)

4. In collaboration with Dr. Paul Black of NIAID, a study is in progress of the chromosome changes that occur when cell cultures of golden hamster cells undergo malignant transformation in vitro following infection with a specific virus, SV-40 (Simian vacuolating virus). In connection with this study, it was necessary to determine the normal chromosome pattern of the golden hamster. As a result of this investigation, it was found that a dispute exists in the literature as to the morphology of the Y chromosome in the hamster. We have been able to resolve this dispute by demonstrating that different strains of golden hamsters are in use in different laboratories throughout the world which differ in the morphology of their Y chromosome.

   Studies of virally transformed cells have shown chromosome changes induced by the SV-40 virus to be indistinguishable from those chromosome changes found in spontaneously occurring malignancies.

Significance to Dental Research:

Most malformation syndromes associated with chromosomal aberrations have widespread abnormalities, including abnormalities of the head, neck, and oral cavity. The study of the chromosomes of other individuals, and their families, where widespread abnormalities are present, may result in the discovery of further syndromes correlated with chromosomal abnormalities.

The study of basic factors involved in cell growth in normal and malignant cells is of obvious importance to medicine and dentistry.

Proposed Course of Project:

All of the investigations described will be pursued further. Publications reporting the results of parts 2, 3, and 4 above will be submitted within the next calendar year. In particular, intensive effort will be given to elucidating the growth-stimulating mechanism of phytohemagglutinin in lymphocyte cultures.

Part B included Yes

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1. Clinical Investigations
2. Human Genetics
3. Bethesda, Maryland

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Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Cooper, Herbert L.: The use of human chromosome markers in linkage studies. Transactions of the N. Y. Academy of Sciences, 24: 383-394, 1962.

Cooper, Herbert L., Kupperman, H.S., Rendon, O. and Hirschhorn, K.: Sex chromosome mosaicism of type XYY/XO. New England Journal of Medicine, 266: 699-702, 1962.

Cooper, Herbert L. and Hirschhorn, Kurt: Enlarged satellites as a familial chromosome marker. Am. J. Human Genet., 14: 107-124, 1962.

Cooper, Herbert L.: Recent advances in human chromosome studies bearing on sex determination. J. National Medical Association, 54: 415-423, 1962.

Honors and awards relating to this project: None.

Serial No. NIDR-80 (C)
1. Clinical Investigations
2. Human Genetics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1962

Part A

Project Title: Hereditary Renal Dysfunction with Associated Anomalies - Genetic and Clinical Studies

Principal Investigators: Dr. G. Cassady, Dr. K. S. Brown

Other Investigators: Mr. M. Cohen, Dr. B. L. Hanna

Cooperating Units: Department of Pediatrics, Duke University Medical Center and Gallaudet College

Man Years (calendar year 1962):

Total:	3/4
Professional:	1/2
Other:	1/4

Project Description:

Objectives:

To study the genetic, clinical and laboratory abnormalities of families with hereditary renal dysfunction.

Methods Employed:

Personal examination and laboratory study of the serum and urine of approximately 500 members of six families have been carried out. Clinical examination of nine members of three of these families has been done, including electroencephalogram, pyelogram, renal biopsy, renal arterogram and detailed audiometric examination. In addition to routine clinical laboratory studies of urine, including electrolytes, urea, uric acid, cholesterol and glucose, special studies of renal physiology and protein and lipoprotein fractions have been carried out. These results have been subjected to electronic data processing in a preliminary attempt to determine the relative diagnostic value of the various procedures.

Patient Material:

Members of families in which this condition has been found. Over five hundred seen in field study, and nine as Clinical Center patients.

Part A (continued)

In-patient hospital days - 144.

Major Findings:

1. A genetic mode of inheritance involving non-random chromosomal segregation has been postulated.

2. The clinical and pathological features of this syndrome have been clarified.

3. Specific biochemical changes in serum lipoproteins have been demonstrated.

4. The nature of the associated hearing loss has been determined.

Significance to Dental Research:

This project indicates that some genetically determined causes of deafness not only involve abnormalities of the auditory mechanism, but abnormalities in the entire oral-oto-facial complex.

Proposed Course of Project:

The project will continue to investigate the genetic, clinical and biochemical factors of this condition. Attempts will be made to determine its frequency in the general population. Further evaluation of the diagnostic value of the many tests used will be carried out. The clinical and laboratory findings are being prepared for publication.

Part B included No

Serial No. NIDR- 81 (C)
1. Clinical Investigations
2. Human Genetics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1962

Part A

Project Title: Hereditary Biochemical Defects of Speech, Hearing, and Mastication

Principal Investigator: Dr. C. J. Witkop, Jr.

Other Investigators: Dr. N. C. Brown, Dr. K. S. Brown, Dr. F. V. Henry, Mr. R. W. Robinette

Cooperating Units: National Institute of Arthritis and Metabolic Diseases

Man Years (calendar year 1962)

Total:	1-1/4
Professional:	3/4
Other:	1/2

Project Description:

Objectives:

To search for possible biochemical defects which may underlie genetically determined hereditary disorders of speech and hearing as have been indicated by the findings in histidinemia, Sjögren-Larsson syndrome and the renal dysfunction and deafness syndrome.

Methods Employed:

Speech and mastication disorders which appear to be hereditary or which appear to be based on a loss of sensory reflex stimulation from oral sensory or proprioceptive stimulation will be the first choice for screening. This is based on observations made in the above-named syndromes.

Speech and masticatory defective populations will be screened on these clinical criteria and also on the basis of the ferric chloride urine test for histidinemia.

Urine samples collected will be further screened by paper chromatography, using a variety of buffers and specific stains for the presence of aminoaciduria or other abnormal urinary constitutents. Serum samples will be subjected to a number of different electrophoretic and

Part A (continued)

chemical analysis for abnormal constituents or patterns.

A battery of clinical neurological and speech tests will be employed to further delineate the functional aspects of these defects.

Patients discovered with specific defects or carriers of the genes will be placed on loading experiments to define the chemical blocks or carrier state. It is planned to investigate the consequences of defined diets on the course of the speech defect in histidinemia.

Patient Material:

Patients will be obtained by referral from the Montgomery County Study, the Haliwa Study and private practicioners. Total numbers are difficult to estimate, but three patients were admitted in 1962, and 25 to 50 can be expected for 1963.

Major Findings:

1. A variable aminoaciduria high in histidine and arginine appears associated with the patients with Sjögren-Larsson syndrome of oligophrenia, ichthyosis and spasticity.

2. In addition to these signs previously described by others, we have noted a speech defect which cannot always be correlated with the degree of oligophrenia and a loss of sensation in the anterior oral cavity, which we believe is the basis of a defect in masticatory function.

3. The description of histidinemia by LaDu, Seegmiller and Howell lead to the postulation by us on the basis of the speech defects in their two patients and three cases from the literature, that this biochemical block would be found to be a specific biochemical defect in speech. This has been substantiated by the fact that, of ten known cases, nine have a speech defect.

4. The prevalence of histidinemia is low, as a screening of speech patients at two large institutions has not revealed a single example of this defect to date.

Significance to Dental Research:

The finding of disorders that appear to be genetically determined biochemical defects and associated with speech and hearing disorders leads to the possibility that these conditions can be treated.

Proposed Course of Project:

One paper on Sjögren-Larsson Syndrome and Histidinemia has been accepted for publication by the Journal of Speech and Hearing Disorders.

Part A (continued)

During the coming year about 3,500 children in special speech and hearing classes in Montgomery County will be screened for these disorders. Selected cases will be admitted to the Clinical Center for detailed clinical study.

Part B included Yes

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1. Clinical Investigations
2. Human Genetics
3. Bethesda, Maryland

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Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Witkop, C.J., Jr., and Henry, F.V.: Sjögren-Larsson syndrome and histidinemia. Hereditary biochemical diseases with defects of speech and oral functions? J. Speech and Hearing Disorders (in press.

Honors and awards relating to this project: None

Serial No. NIDR-82 (C)
1. Clinical Investigations
2. Oral Pharyngeal Development Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1962

Part A

Project Title: Clinical Studies of Oral and Pharyngeal Disabilities Resulting from Local Anomaly, Neurological Impairment, Neoplasm or Surgical Excision

Principal Investigator: Dr. J. F. Bosma

Other Investigators:
Dr. P. J. Coccaro
Dr. Y. Takagi
Dr. J. L. Ackerman
Dr. J. M. Lifschiz
Dr. F. V. Henry
Dr. E. B. Miner
Dr. W. H. Richmond

Cooperating Units:
Dental Clinic, Department of Radiology, Clinical Center
National Cancer Institute
Memorial Cancer Hospital, New York
Integrated Cleft Palate Clinic, Johns Hopkins Hospitals, Baltimore, Maryland

Man Years:

Total: 3 3/4

Professional: 2 3/4

Other: 1

Project Description:

Objectives:

1. Further understanding of anatomical development and abnormalities of form and dimension of skeleton and musculature.

2. Further understanding of physiological development and abnormal mechanisms of feeding, upper respiratory and speech functions.

Part A (continued)

Methods Employed:

1. Cineradiography - laterally directed cineradiography focused at mid-mouth, mesopharynx or hypopharynx. Visualization of soft structures in motion facilitated by metal tags glued to standard sites on surface of lips, tongue or palate and by radiopaque media in feeding or at previous administration. Subject performance in upright or supine position, and in practiced actions.

2. Cinematography

3. Sound recording - by microphone in air or applied to skin of neck. Multi-channel tape recording. Later analysis of sounds by ear and of selected portions by spectrographic analysis (Sonagraph).

4. Respiratory pressure recording - from esophagus or trachea.

5. Electromyography from surface applications on oral mucosa or skin.

Patient Material:

Subjects - Infants, children and adults having anomalies such as cleft palate, hypoplasia of tongue, hypoplasia and/or displacement of mandible, etc. For intensive study, young subjects having severe deformities, such as cebocephaly or cleft face are admitted as inpatients in the Clinical Center. Persons having impairment of feeding and/or respiratory functions associated with neurological disease, such as athetotic or spastic dyskinesia. Subjects having maxillo-facial surgical excisions for removal of neoplasms.

Major Findings:

1. Standard descriptions of mature motor performances of oral and pharyngeal actions in feeding and in speech are generally validated but grossly extended in variety. These variations in motor pattern are found without significant variation in form or proportions of skeleton or soft tissues.

2. When anomalies or lesser gradations of structural form are present, these variations of motions are greater still, particularly in the performances of speech. A significant element in these variant performances in normal or in anomalous circumstance, is that adequacy is usually achieved in particular elements of function. In example, pharyngeal airway is maintained

Part A (continued)

in adequacy, palato-pharyngeal closure is achieved at onset of swallow and during orally-emitted vocalization, and the action of swallow fully empties the pharynx.

3. In neurologically impaired subjects, however, the motor performances of feeding, airway maintainance and speech are impaired, by disability of their sensory elicitations, of their central coordinations or of their motor effector system. In this project study, particular attention is given to these separable categories of neurological performance. For example, the sensory elicitation systems are under study in cleft palate subjects.

Significance to Dental Research:

Further definition of mechanisms of disability and of the reciprocal influences of abnormality of form upon function and abnormality of function upon form.

Proposed Course of Project:

Continuing development of methods. Extensions of these clinical studies.

Part B included: No

Serial No. NIDR-83 (C)
1. Clinical Investigations
2. Oral and Pharyngeal Development Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1962

Part A

Project Title: Studies on the Pharyngeal Receptor Systems Affecting Fragmental Regulation of Respiration

Principal Investigator: Dr. Y. Takagi

Other Investigators: Dr. J. F. Bosma
Dr. J. V. Irwin
Dr. J. M. Lifschiz

Cooperating Units: None

Man Years:

Total: 1 5/6

Professional: 1 1/3

Other: 1/2

Project Description:

Objectives:

The respiratory regulation has been attributed to such factors as:

1. Chemical changes within the circulatory system

2. Changes in hemodynamics

3. Physical and mechanical stimuli acting on various peripheral receptor systems. In general, it has been held that these factors establish and modify the depth and direction of respiratory cycles.

There are, however, within the respiratory system, localized sites which can elicit either exaggerated inspiration or expiration. The former, which may be described as sniffing, is induced by stimulation of the mucous membrane of the nasal cavity. The latter, which is a phenomenon of coughing, is elicited by stimulating the mucous

Part A (continued)

membranes of the larynx and trachea. The present study was primarily initiated in order to investigate the gradation of this control. This study was also provoked by observations of notably varied mandibular and lingual air compression actions of the infant in natal transition. It is possible that the pharynx constitutes the principal site of perception of the changed physical environment at birth. These studies were also in part motivated by observations in the field of speech physiology in which the relevance of epipharyngeal sensation to the palatopharyngeal closure in speech articulation has been noted.

Methods Employed:

Electrical stimulations of the dorsal pharyngeal wall and its adjacent structures were performed on cats and dogs (total number is approximately 80) under Urethane anesthesia. Dorsal pharyngeal wall was surgically exposed. The effect of stimuli was evaluated by recording the intra-tracheal pressure variation, electromyography of the diaphragm, intrinsic muscles of the vocal cord, and the musculature of the tongue, palate, pharynx and submental region. In addition to these methods, the recording of the vocal cord motion by cinematography was attempted in two cats and utilization of light penetration method was also successful.

Major Findings:

The electrical stimulation of the dorsal wall of the upper pharynx elicits qualitatively different variations in intra-tracheal pressure than do those applied at the lower pharynx. Stimulation of the upper pharynx with slightly supra-threshold stimuli resulted in the initial appearance of the effect in expiration rather than adjacent to the terminating or beginning portions of inspiration. These penetration phenomena also demonstrated lesser degree of adaptation, and in many occasions the effect of stimuli was actually cumulative. The stimuli applied upon the lower pharynx resulted in the deflection of intra-tracheal pressure during the terminating portion of inspiration rather than during the phase of expiration. These phenomena demonstrated marked adaptation. The electromyographic manifestation of these stimuli was the brief moment of inhibition of the muscle which was participating in respiration. With the increase in stimuli intensity, there were brief pulses of activation of the counteracting muscle associated with brief period of inhibition of the actively participating respiratory muscles at the moment of stimulation. With excess of stimulation, there were separable pulses of activation observed resulting in co-contraction of the respiratory muscles. The latency of these responses was 16 to 28 milli-seconds indicating that these responses are multi-synaptic.

Part A (continued)

Significance to the Dental Research:

Certain orthodontic problems, such as severe malocclusion, complete inlocking of the mandible, retruded mandible, and various disorders involving cranio-facial regions present unique problems of "displacement of the tongue." Whether the displacement of the tongue is primary or secondary to the deformity of the mandible is a matter of opinion. Yet, here we are demonstrating particular receptor systems lying within the air passage, which may regulate position of the tongue, hyoid, mandible and palate to maintain the patency of the airway. In cases such as the Pierre Robin Syndrome, this system may be deficient or hypoplastic, thus presenting a unique problem of dysphagia and respiratory distress.

Proposed Course of Study:

To trace this reflex pathway centrally. Also to search out the similar reflex mechanisms in the normal human and in those with craniofacial disorders.

Part B included: Yes

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Part B: Honors, Awards and Publications

Publications other than abstracts from this project:

Bosma, J. F., Irwin, J. V., Lifschiz, J. M., and Takagi, Y. "The Effect of Electrical Stimulation of the Pharynx on Respiratory Regulation." In preparation.

Honors and Awards Relating to this project: None

Serial No. NIDR-84 (C)
1. Clinical Investigations
2. Oral Pharyngeal Development Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1962

Part A

Project Title: Behavior of Points* "A" and "B" in Immediate Denture Patients Utilizing Tantalum Pins

Principal Investigator: Dr. P. J. Coccaro

Other Investigators: None

Cooperating Units: None

Man Years:

Total: 1

Professional: 1/2

Other 1/2

Project Description:

Objectives:

Studies have shown that there is a good chance that maxillary and mandibular apical base relationships, in the region of the central incisors, can be improved through orthodontic treatment. General acceptance of the fact that an intimate relationship exists between points A and B and the apices of the maxillary and mandibular central incisors has created the conception that these points are notably affected by the alterations in the position of the root apices of the maxillary and mandibular central incisors.

* Point A - Subspinale: The deepest midline point on the maxilla between the anterior nasal spine and prosthion.

Point B - Supramentale - the deepest midline point on the mandible between infradentale and pogonion.

The location of the A-B plane in relation to the facial plane is a measure of the relation of the anterior limit of the denture bases to each other and to the profile.

Part A (continued)

Thus the basic purpose of this study was to evaluate the behavior of point A and point B, with the aid of metallic pins in immediate denture patients who had their maxillary and mandibular central incisors removed.

Methods Employed:

1. The subjects included in this study were divided into two groups varying in number and treatment. The smaller group consisted of two subjects, one male age forty-six, and one female, age thirty-seven. Each subject had tantalum pins inserted into the areas usually selected as cephalometric landmarks used to identify points A and B as previously defined. These patients received immediate full upper and lower dentures following surgery. In addition a larger group of twenty-two subjects (seventeen males and five females) having an average age of forty-one years was also studied. None of these individuals had metallic pin implants or immediate dentures. Their predenture insertion records showed an average of 3.6 months. The conventional method of selecting point A and point B was employed.

   Subjects in both groups had all of their remaining teeth removed under general anesthesia. The smaller group (two subjects) was treated in the following manner: After being satisfactorily anesthetized, vertical incisions were made in the cuspid region, bilaterally. A mucoperiosteal flap was peeled back to completely expose the labial plate of bone and thus permit direct access to the points under investigation. In order to implant the tantalum pins an 18 gauge spinal needle was employed. It was two inches long with a stylet (diameter 0.75 mm). The length of the cannulae was shortened to permit the stylet to project 2 mm beyond the end of the cannulae. A tantalum pin (diameter 0.75 mm - length 2 mm) was inserted into the pointed end of the cannulae which was then positioned against the bone in the area of point A and point B. Following this the stylet was inserted into the cannulae until it contacted the pin; the stylet was tapped with a mallet to drive the pin into the bone.

   Removal of maxillary and mandibular incisor teeth was completed and the microperiosteal flaps were sutured back into position. Full upper and lower dentures were inserted immediately following surgery.

   Lateral cephalometric x-rays were taken on all patients at the following periods: Pre-surgery - Denture Insertion - and at 6 and 12 months following insertion of dentures.

Part A (continued)

To discern antero-posterior changes at point A and point B angular measurements were used; angle SNA* (sella-nasion-point A) was used for the former and angle GPB* (gonion-pogonion-point B) for the latter. Linear measurements were used to evaluate supero-inferior changes at point A and point B; this was accomplished by dropping perpendicular lines from S-N (sella-nasion-plane) to point A and from MP* (mandibular plane) to point B.

Major Findings:

1. Angular measurements for Point A during the period between pre-surgery and twelve months following surgery indicated that the subjects in both groups showed point A to locate more posteriorly twelve months following surgery - this was true for both groups studied. On a comparative basis point B appeared to locate more posteriorly twelve months following surgery than did point A. This also was true for both groups.

2. Linear measurements indicated that point A, during the interim between pre-surgery and twelve months following surgery, tended to locate slightly higher (for the "pin" cases) and slightly lower (for the larger group). Point B, on the other hand, during the same period, located higher in one "pin" case and lower in the other. The mean for the larger group showed a lowering of point B.

Significance to Dental Research:

1. The use of point A and point B in the analysis of the skeletal pattern for patients undergoing orthodontic treatment is well known - nevertheless, the location and stability of point A and point B continues to be the subject of much criticism.

   It may appear from these findings that point A is a fairly reliable anterior landmark on the maxilla and more closely associated with basal bone. Point B, on the other hand, is more

* (S) Sella - midpoint of sella turcia determined by inspection.
(N) Nasion - most anterior point of the naso-frontal suture seen from norma lateralis.
(G) Gonion - is obtained by bisecting the angle formed by the tangents to the lower and posterior borders of the mandible.
(P) Pogonion: most anterior point of bony chin.
(MP) Mandibular Plane: A line at the lower border of the mandible tangent to the gonion angle and the profile image of the symphsis.

Part A (continued)

superiorly located and appears to become closely related to the alveolar process of the mandible - thus we see more notable change about its location.

2. Contributes added information relative to the stability of the points under investigation thus permitting such points to be used more judiciously in the skeletal and dental analysis of patients undergoing orthodontic treatment.

Proposed Course of Project:

1. Plan to utilize the two subjects with tantalum pin implants for a serial study of the changes in occlusal vertical dimension from pre-extraction period to twenty-four months following surgery. With the "pins" as fixed landmarks we may be able to appraise, on a longitudinal basis, changes occurring within the upper, middle and lower face.

Part B included: No

Serial No. NIDR-85 (C)
1. Clinical Investigations
2. Oral Pharyngeal Development Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1962

Part A

Project Title: Longitudinal Study of Soft Tissue Profile in Children with Unilateral Cleft Lip and Palate

Principal Investigator: Dr. P. J. Coccaro

Other Investigators: None

Cooperating Units: University of Illinois, Cleft Palate Center
Chicago Professional College

Man Years:

Total: 1

Professional: 1/2

Other: 1/2

Project Description:

Objectives:

1. To study the nature of the soft tissue profile in patients with a cleft of the lip and palate, during their early years of development.

2. To analyze growth of the soft tissue profile in the region of the upper lip and the nose.

3. To determine, on a longitudinal basis, how the skeletal and soft tissue profile of cleft palate subjects compares with that of normal subjects.

Methods Employed:

1. The data selected for this study was derived from the serial growth study at the Cleft Palate Center of the University of Illinois. Serial cephalometric roentgenographic records taken on 21 subjects from 3 months to 7 years of age were studied. The sample included fourteen males and seven females. All subjects had a unilateral cleft of the lip and palate. Eleven had

Part A (continued)

an involvement on the left side of the face while ten subjects had the right side affected.

Comparisons were made with normative data, published by Subtelny, on the soft tissue profile analysis.

2. A total of approximately two-hundred lateral roentgenograms were traced and analyzed. The nose, upper and lower lips, the chin and the soft tissue overlying the mid-frontal region were traced from each lateral head plate.

   Cranial landmarks, such as basion, BA, the middle of the anterior margin of foramen magnum and nasion, N, the anterior-most point of the junction of the frontal and nasal bones were also traced. Other skeletal landmarks employed were (point A), the deepest midline point on the pre-maxilla between anterior nasal spine and prosthion, and (P) pogonion, the most anterior point on the mandible in the midline.

   Accpetable methods of superimposition and measurement were employed to evaluate changes on a time basis.

Major Findings:

1. Prognathism of Skeletal Chin - The skeletal chin tended to become more prognathic with increment in age. A notable increase was observed after the second year which continued through the fifth year of life. During the interim between the fifth and seventh years, a decrease in prognathism was recorded. During the periods studied, the tendency toward increased mandibular prognathism - though quite apparent - was notably less for the cleft palate group (2.1^{o}) compared to the normal male group (4.6^{o}).

2. Prognathism of Integumental Chin - The integumental chin also tends to become more prognathic with advancing age.

   When the cleft palate group was compared to the normal group it was apparent that the former had an integumental chin which was less prognathic at every age level studied.

3. Skeletal and Soft Tissue Mandibular Prognathism - The same pattern of change was noted in both skeletal and soft tissue profile measurements for the cleft palate group. When compared to normative data, the cleft palate group revealed a skeletal and integumental chin that was more retrusive at every age level studied; i.e. from birth through seven years of age.

Part A (continued)

4. Convexity of Skeletal Profile - Average angular measurements reveal a rapid decrease in the convexity of the skeletal profile from six months to three years of age. During the first five years of life, there is apparent straightening of the cleft palate skeletal profile. Some slight increase is noted in skeletal profile convexity between the fifth and seventh years.

   When compared to normative data a greater degree of skeletal facial convexity is noted at every age level for the cleft palate subjects.

   A greater decrease in convexity of the skeletal profile was noted for the normal group over the cleft palate group during the periods studied.

5. Convexity of Soft Tissue Profile - Two methods were employed to evaluate changes in the convexity of the soft tissue profile. The first included the profile formed by the soft tissue areas, NS - SN - PS, which are defined as follows: NS, obtained by continuing the cranial base line anteriorly to intersect the soft tissue profile. This point of intersection was designated as NS - soft tissue nasion, SN, subnasale, is the point where the lower margin of the nasal septum is confluent with the integumental upper lip, soft tissue pogonion, PS, is described as the most anterior point of the soft tissue chin. The second method employed the tip of the nose as the central point of the angle and permitted an evaluation of the soft tissue profile convexity using this most ventrad soft tissue profile projection.

   Thus, average measurements of the convexity of the soft tissue profile, excluding the nose, were found to show a reduction in the profile convexity. The soft tissue profile tended to become straighter from six months to six years of age. The cleft palate group tends to reflect a soft tissue profile that is less convex, at most ages, than that recorded for the normal group.

   Averaged angular measurements of the convexity of the soft tissue profile, including the nose, tended to become less convex up to the fourth year, after which time the profile becomes more convex.

   When compared to the normal, the cleft palate group reflects a soft tissue profile, including the nose, that is less convex from six months to seven years.

Part A (continued)

Significance to Dental Research:

A thorough assessment of the soft tissue profile, on a longitudinal basis, may enhance our knowledge of the status and growth characteristics of specific areas that are responsible for the formation of the facial profile. It may permit plastic surgeons and orthodontists to formulate more effective time schedules for the essential surgical and orthodontic treatment procedures periodically needed for rehabilitation of these patients.

Proposed Course of Project:

A further study is in progress on a group of cleft palate subjects, who had no areas of involvements in the soft tissue profile. A comparative analysis will be made between the two groups.

Part B included: Yes

PHS-NIH
Individual Project Report
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Part B: Honors, Awards and Publications

Publications other than abstracts from this project:

Coccaro, P. J., Subtelny, J. D., Pruzansky, S. Growth of Soft Palate in Cleft Palate Children - Plastic and Reconstructive Surgery, Vol. 30, No. 1. 43-55. 1962.

Honors and Awards Relating to this Project: None

Serial No. NIDR-86 (C)
1. Clinical Investigations
2. Oral Pharyngeal Development Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1962

Part A

Project Title: Brain Stem Mechanisms in the Integration of Reflex Deglutition

Principal Investigator: Dr. W. H. Richmond

Other Investigators: None

Cooperating Units: None

Man Years:

Total: 1

Professional: 1/2

Other: 1/2

Project Description:

Objectives:

It is the specific purpose of this phase of the project to define the exact neuroanatomical areas of the brain stem that subserve reflex deglutition.

Methods Employed:

1. Electrophysiological: Single unit (cell) firing during deglutition is recorded with microelectrodes in the medulla oblongata. The motor expression of swallowing is monitored electromyographically. The activity of these single units is observed before and after flaxedilization and hemisections of the brain stem.

Major Findings:

1. Non-respiratory neurons in the dorso-lateral reticular formation of the medulla (nucleus reticularis parvocellulous) show electrical activity synchronous with swallowing. The significance of this, if it can be confirmed that these are swallowing neurons, is that it indicates the central synapse of swallowing afferents is outside of nucleus of fasciculus solitarius.

Part A (continued)

2. Expiratory neurons in the same region show cessation of electrical activity during swallowing. This suggests that during swallowing respiration is inhibited both in inspiration and expiration.

Significance to Dental Research:

This work is relevant to the field of oral-pharyngeal physiology.

Proposed Course of Study:

1. Confirmation of above findings in other species.

2. Tracing pathways from the proposed swallowing center to motor nuclei.

3. Investigation into the physiological mechanisms that trigger the swallowing center; specifically the significance of change in steady and generator D.C. potentials.

Part B included: No

Serial No. NIDR-87 (C)
1. Clinical Investigations
2. Oral Pharyngeal Development Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1962

Part A

Project Title: Analysis of the Development of Facial and Cranial Skeleton in Mammals as Revealed by Vital Staining.

Principal Investigator: Dr. M. J. Baer

Other Investigators: None

Cooperating Units: None

Man Years:

Total: 1 3/4

Professional: 1/2

Other: 1 1/4

Project Description:

Objectives:

1. To develop a technique of vital staining using two or more colors of dye in order to facilitate the detailed discrimination of the sites of bone deposition and resorption in the mammalian skull.

2. To determine the role played by differential apposition and resorption in changing the shapes of the individual bones in the face and cranium during ontogenetic development.

Methods Employed:

Rats and rabbits of known age and sex are injected with alizarin red "S" and acid alizarin blue BB at fixed time intervals during the first 125 days of life. The animals are sacrificed at varying time intervals in order to demonstrate the growth of new unstained bone which contrasts with the older bone stained by the brief exposure to the injected dye. Skulls are prepared by gross maceration and the enzymatic (papain) removal of soft tissues. Clean skulls of animals over ten days of age at sacrifice are imbedded in a plastic

Part A (continued)

matrix to facilitate undecalcified sectioning (90 micron sections) on a thin section saw. Animals under ten days of age are sectioned through the use of conventional histological techniques of wax imbedding and microtome cutting. The mounted sections are examined to determine the sites of deposition of the vital stains.

Major Findings:

Examination of the skulls of rats and rabbits submitted to the methodological approach indicated above demonstrates the feasibility of vital staining growing bone with dyes of at least two colors. The dye, acid alizarin blue BB, is absorbed into growing bone in a manner comparable to alizarin red "S." Animals injected first with alizarin red "S" and then subsequently injected with acid alizarin blue BB after a time interval, depending upon the age and rate of growth of the animal, reveal layers of red, white and blue bone indicating the bone laid down at the times of the absorption of the dyes and the intervening unstained bone formed between the time of the two injections. The use of dyes of different colors promises to facilitate the analysis of sites of successive bone deposition and resorption in a manner not possible through the use of a single dye.

Significance to Dental Research:

Further definition of mechanisms determining mammalian skeletal form and dimension is of basic significance to those clinical fields concerned with the skeleton and with body form.

Proposed Course of Project:

1. Extension of methods to pig and to a representative carnivore.

2. Analysis and homologic comparisons of stain marked skeletons.

3. Imposition of developmental distortion by surgical removal of different muscle complexes in order to determine the resultant effect on the pattern of bone development.

Part B included: No

Serial No. NIDR-88 (C)
1. Clinical Investigations
2. Oral Pharyngeal Development Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
January-August 1962

Part A

Project Title: The Sensory Experience of the Mucosal Surfaces of the Oral Cavity.

Principal Investigator: Dr. R. C. Grossman

Other Investigators: Dr. J. F. Bosma
Dr. J. M. Lifschiz

Cooperating Units: Clinical Center Dental Department, NIH

Man Years:

Total: 1

Professional: 1/2

Other: 1/2

Project Description:

Objectives:

1. To design instruments for the quantitative evaluation of sensory experience within the oral cavity.

2. To obtain initial normative data using these devices and to employ this information and instrumentation in diagnosing oral sensory deficiencies.

3. To develop a "sensory prosthesis" to provide supplemental sensation during speech articulations in patients exhibiting such deficits.

4. To review the literature pertaining to the neurohistology of sensory terminations in the oral mucosa and to relate this information to observations from current clinical research.

Part A (continued)

Methods Employed:

1. For assessing tactile experience of the mucosal surfaces, a single probe is jointed at the center of rotation of a precision dynamometer to facilitate uniform and slight force upon contact with the tissue. The probe is also used for electrical stimulation of the mucosae with monopolar or bipolar electrode tips. This is desirable because electrical stimuli are more readily controlled than other means of eliciting sensation. Galvanic current, which frequently causes responses appropriate to taste, is not used in these studies. DC pulses effectively elicit the other modalities (pain, temperature, and touch) perceived in the mucous membrane, although such stimulation may not excite these modalities to the same degree and may even stimulate proprioceptive receptors. A standard laboratory stimulator provides DC pulses of controlled frequency, duration, and voltage; and these may be monitored on a cathode ray oscilloscope. For accurate quantification of test stimuli, it is advisable to use a regulated current, because tissue resistance varies, and it is the amperage, and not voltage, which stimulates receptors. Two point discriminatory ability may be assessed with an instrument composed of dual probes on a modified pair of bow dividers which is attached to the deflection arm of a precision dynamometer. Replacing the dual probe tips with a multiple pointed rotating disc provides a method of evaluating differences in site discrimination and spatial summation in response to tactile and electrical stimuli which is similar to that used in neurological examination of the skin.

   Stimulus parameters required to elicit subject responses with these instruments are represented numerically at the sites of stimulation on diagrammatic projections of the oral mucosal surfaces.

2. The instruments described above were employed in determining the normal range of sensory experience within the human oral cavity. In addition, subjects were asked to identify geometrically shaped, unknown objects placed in the mouth. Selective blocking of sensation in various areas of the mouth was accomplished during the latter studies by means of lingual anesthesia and palatal prostheses.

   These methods were used to assess the degree of oral sensory deficiency in a fifteen-year-old female exhibiting dysarthria and oral dysphagia.

Part A (continued)

3. An instrument has been developed to provide electrical stimulation for persons deficient in oral surface perception. This device consists of a thin palatal prosthesis, a relay activator circuit, and a standard laboratory stimulator. Contact of the tongue with a trigger electrode on the lingual surface of the prosthesis completes the relay circuit. This activates the stimulator to provide DC pulses of controlled duration, frequency, and voltage to a stimulating electrode on the palatal surface of the prosthesis as long as the tongue is in contact with the trigger electrode. The stimulus is monitored on a cathode ray oscilloscope. A small electric lamp further indicates the duration of each tongue-to-palate contact and can be used as reinforcement of the electrical stimulus in conditioning the subject to such supplemented sensory experience.

   Stimulation of the palate is at the rate of 100 pulses per second, with a pulse duration of one millisecond; and the voltage is adjusted to provide a regulated current that will slightly exceed the threshold level of electrical sensation. The latter varies with the degree of sensory deficit of the area under consideration. In initial use of this device only single electrode pairs have been employed. However, multiple stimulator units with electrode pairs appropriately oriented in the prosthesis could permit the subject to experience contact of the tongue with sites in the hard palate adequate for several speech articulations.

4. An extensive survey of the literature pertaining to the sensory receptors of the oral mucosa has been partially completed. This task has been made quite complex by the perpetuation of confusion in this literature. This has been caused by arbitrary distinctions between transitional forms, apparent differences in microscopic details due to the multiplicity of tissue preparation methods, and by reluctance to depart from tradition. Thus an inordinately large number of supposedly different sensory endings has been reported during the past century. The application of descriptive terminology to the neural elements in the body surfaces has simultaneously clarified our interpretations of the neurohistology of the oral mucous membrane.

Patient Material:

Several young adult individuals were studied for the accumulation of normative data. Five subjects exhibiting neurological disorders with oral manifestations in the form of sensory deficiency were also studied. These persons, four of whom were females, ranged in age from six to twenty years of age.

Part A (continued)

Major Findings:

1. Studies employing tactile and electrical stimulation indicate a progressive decrease in sensory capacity toward the rear of the mouth. The rugae are found to be much more sensitive than other areas of the palate; and the lip is also more sensitive to tactile stimulation than areas of the buccal mucosa situated more posteriorly. The same is true of the dorsum of the tongue from the tip toward the rear. Two point discrimination assessments at the tip of the tongue and the anterior of the dorsum are far more critical than at the lateral margins and toward the rear. The latter areas require twice the distance between points of contact in order to permit their identification as two separate contacts. The oral recognition of geometrically shaped objects confirms the foregoing findings: It has been observed that the lips, anterior of the tongue dorsum, and anterior of the palate are the most sensitive and critical areas in such discriminations.

2. An attempt to simplify the complexity of terminology and interpretation in the literature has resulted in three broad classifications of oral mucosal sensory terminations: Organized endings (after the definition of Sinclair, Weddell, and Zander, 1952), free nerve endings, and nerve networks of varying degrees of complexity. This clarification reveals that there is a progressive decrease in the frequency of specific nerve endings from the front to the rear of the mouth which has not been recognized previously and which supports the observations noted in the previous paragraph.

3. Early results in this investigation indicate that a subject who previously experienced no sensation with tongue-to-palate contacts can feel such appositions with the assistance of the sensory prosthesis.

Sifnificance to Dental Research:

The variety of instruments and techniques developed and their manner of application to various aspects of the neurological examination provides a new parameter to investigations of the sensory cues to motor activities in the oral and pharyngeal region. Thus, more specific description of stimulus characteristics than was previously available from conventional neurological testing instruments is now possible. The information derived as a result of these developments has an important bearing on the investigations of the Oral and Pharyngeal Development Section pertaining to the elicitations and reflexes involved in swallow, cry, respiration, cough, laryngospasm,

Part A (continued)

mastication and tongue thrust. Furthermore, this approach to the study of sensory deficiencies in the mouth is basic to subsequent research in the field of speech pathology.

Proposed Course of Project:

This work will continue jointly in the Oral and Pharyngeal Development Section, NIDR, under the direction of Dr. Bosma as well as on an extramural basis at the School of Dentistry, University of California at Los Angeles, under the direction of the principal investigator. Techniques and instrumentation for tactile and electrical stimulation will be refined and enlarged upon to include assessments of the modalities subserving thermal sensation.

Part B included: Yes

PHS-NIH
Individual Project Report
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Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Grossman, R. C. and Bosma, J. F. Instruments for Evaluating Surface Sensory Experience in the Mouth. Journal of Dental Research. In Press. 1962.

2. Grossman, R. C. and Bosma, J. F. An Experimental Oral Sensory Prosthesis. Journal of Dental Research. In Press. 1962.

Honors and Awards Relating to this Project: None

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